EVALUATION OF BACTERIAL AND FUNGAL AGENTS IN THE AETIOLOGY OF CHRONIC SUPPURATIVE OTITIS MEDIA WITH SPECIAL REFERENCE TO ANTIMICROBIAL RESISTANCE PATTERN OF *PSEUDOMONAS SPECIES*

Dissertation submitted to

THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY

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for the award of the degree of

M.D.(MICROBIOLOGY)

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CERTIFICATE

This is to certify that this Dissertation entitled **"EVALUATION OF BACTERIAL AND FUNGAL AGENTS IN THE AETIOLOGY OF CHRONIC SUPPURATIVE OTITIS MEDIA WITH SPECIAL REFERENCE TO ANTIMICROBIAL RESISTANCE PATTERN OF *PSEUDOMONAS SPECIES*"** is a bonafide record of work done by **Dr.SANGEETHA BASKARAN,** during the period of her post graduate study from 2013 to 2016 under guidance and supervision in the Institute of Microbiology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai – 600 003, in partial fulfillment of the requirement for **M.D.MICROBIOLOGY** degree Examination of The Tamilnadu Dr.M.G.R. Medical University to be held in April 2016.

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DECLARATION

I declare that the dissertation entitled **"EVALUATION OF BACTERIAL AND FUNGAL AGENTS IN THE AETIOLOGY OF CHRONIC SUPPURATIVE OTITIS MEDIA WITH SPECIAL REFERENCE TO ANTIMICROBIAL RESISTANCE PATTERN OF *PSEUDOMONAS SPECIES*"** submitted by me for the degree of M.D. is the record work carried out by me during the period of **October 2014 to August 2016** under the guidance of **Prof.Dr.U.UMADEVI M.D.** Profess or of Microbiology, Institute of Microbiology, Madras Medical College, Chennai. This dissertation is submitted to the Tamilnadu Dr.M.G.R Medical University, Chennai, in partial fulfillment of the University regulations for the award of degree of M.D. Microbiology (Branch IV) examination to be held in April 2016.

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I humbly submit this work to the Almighty who has given the health and ability to pass through all the difficulties in the compilation and proclamation of this blue print.

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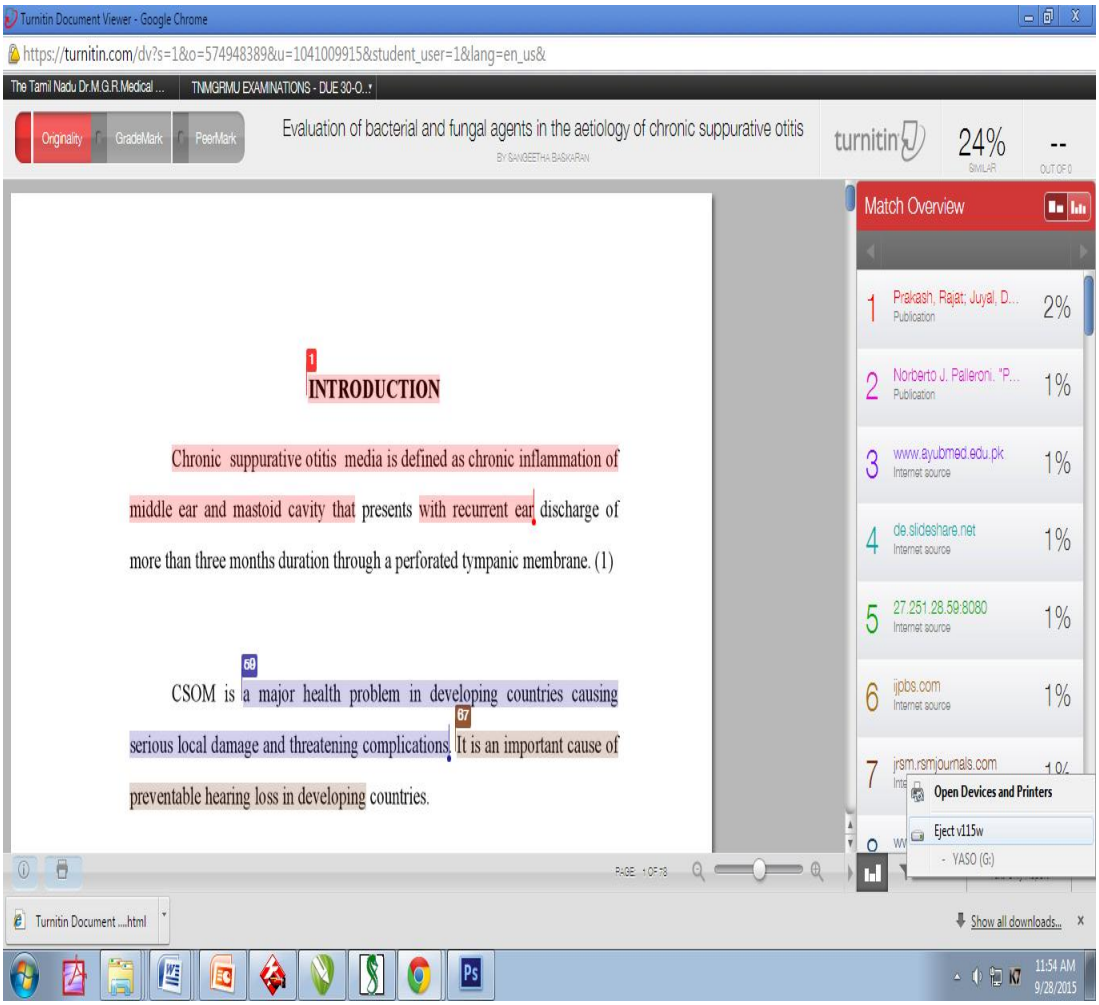
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INTRODUCTION
Chronic suppurative otitis media is defined as chronic inflammation of middle ear and mastoid cavity that presents with recurrent ear discharge of more than three months duration through a perforated tympanic membrane. (1)
CSOM is a major health problem in developing countries causing serious local damage and threatening complications. It is an important cause of preventable hearing loss in developing countries.
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ABSTRACT

Introduction:

Chronic suppurative otitis media is defined as chronic inflammation of middle ear and mastoid cavity that presents with recurrent ear discharge of more than three months duration through a perforated tympanic membrane.

CSOM is a major health problem in developing countries causing serious local damage and threatening complications. It is an important cause of preventable hearing loss in developing countries.

Evaluation of the microbiological agents and their antimicrobial sensitivity pattern in that region is helpful in the initiation of specific therapy and thus minimizing its complications and emergence of resistant strains.

Aim:

To determine the microbiological profile and their antimicrobial sensitivity pattern in patients with chronic suppurative otitis media, with special reference to antimicrobial resistance patterns of *Pseudomonas species.*

Materials and Methods:

This study was conducted at the Institute of Microbiology ,Madras Medical college, Chennai . A total of 100 patients with clinical diagnosis of CSOM from the Upgraded Institute of Otorhinolaryngology were enrolled in the study and the samples were obtained from each patient using sterile cotton swabs and processed by standard microbiological techniques. Detection of Extended spectrum betalactamases

(ESBL),AmpC betalactamases and Metallo betalactamases in Pseudomonas isolates were done by phenotypic methods.

Results:

Analysis of bacterial flora of the present study showed predominance of Gram negative bacilli. The highest incidence was that of *Pseudomonas aeruginosa* (39.6%) followed by Staphylococcus aureus(31.68%). In the present study for all the isolates Amikacin was found to be the most effective drug followed by ciprofloxacin and gentamicin. Antimicrobial susceptibility of Pseudomonas aeruginosa revealed 100% sensitive to imipenem,97% sensitive to piperazillin-tazobactum,78% to amikacin ,75% to ciprofloxacin , 68% to gentamicin and 46% to ceftazidime.

ESBL producers in Pseudomonas aeruginosa was found to be 17.07%,AmpC betalactamase was found in 9.75% .

Conclusion:

The emergence of P. aeruginosa possessing combinations of β-lactamases like ESBL and AmpC betalactamases is a major public health concern necessitating efficient detection and intervention to control drug resistance. Hence continuous and periodic evaluation of microbiological pattern and antibiotic sensitivity is essential to reduce the potential risk of complications and emergence of resistant strains.

INTRODUCTION

Chronic suppurative otitis media is defined as chronic inflammation of middle ear and mastoid cavity that presents with recurrent ear discharge of more than three months duration through a perforated tympanic membrane. [(1)]

CSOM is a major health problem in developing countries causing serious local damage and threatening complications. It is an important cause of preventable hearing loss in developing countries.

Incidence of this disease is higher among people with low socio-economic status because of malnutrition, overcrowding, poor hygiene, inadequate health care, and recurrent upper respiratory tract infection.

It is a massive health problem and India is one of the countries with highest CSOM prevalence (> 4%) where urgent attention is needed.[(1)] It causes conductive and sensorineural hearing loss and has got adverse effect on childhood development. [(2)] It is a common cause of hearing impairment and can occasionally lead to fatal intracranial complications. Hence early and accurate diagnosis of CSOM is life saving.

Both Gram positive and Gram negative bacteria are responsible for infection of the middle ear in addition to fungal etiological agents. Due to advent of newer antibiotics, the microbial organisms and their resistance pattern is changing constantly.

Evaluation of the microbiological agents and their antibiotic sensitivity pattern in that region is helpful in the initiation of specific therapy and thus minimizing its complications and emergence of resistant strains.

Due to the long period of morbidity of CSOM and the repeated occurrences of otorrhea during that period, patients are often prescribed empiric antibiotics in outpatient clinics without microbiologic evaluation. CSOM has received considerable attention not only because of its high incidence and chronicity but also because of issues such as bacterial resistance and ototoxicity with both topical and systemic antibiotics .

The most common microorganisms found in CSOM are *Pseudomonas aeruginosa, Staphylococcus aureus, Klebseilla pneumoniae, Proteus mirabilis, Escherichia coli, Aspergillus spp, Candida spp* and these vary in different geographical distributions.[4] Fungal infections of the middle ear are common as fungi thrive well in moist pus.

Therefore, this study was undertaken to know the pattern of microbes and their antimicrobial susceptibility pattern to provide a guideline for empirical antibiotic therapy.

Pseudomonas aeruginosa, one of the most common organism isolated in CSOM is an important cause of nosocomial infection which has developed resistance to many potent antibiotics .Hence the antimicrobial resistance pattern of *Pseudomonas species* was also analysed in this study.

Though the treatment of CSOM is controversial, and subject to change particularly in developing countries, the antibiogram of these organisms have been reported to vary with time and geographical area, probably due to use and abuse of antibiotics. Hence the need for periodic update of antibiogram for effective chemotherapy and management of CSOM cannot be overemphasized.

AIMS AND OBJECTIVES

AIMS

To determine the Microbiological profile and their antimicrobial sensitivity pattern in patients with chronic suppurative otitis media, with special reference to antimicrobial resistance patterns of *Pseudomonas species* .

OBJECTIVES

1. To isolate aerobic bacterial and fungal agents causing CSOM.
2. To identify the antimicrobial susceptibility pattern of the bacterial isolates.
3. To determine the resistance pattern of *Pseudomonas species*
4. To detect Extended spectrum betalactamases (ESBL),AmpC betalactamases and Metallo betalactamases in Pseudomonas isolates by phenotypic methods.
4. To correlate the association of risk factors with Chronic suppurative otitis media.

REVIEW OF LITERATURE

DEFINITION

Chronic suppurative otitis media (CSOM) is defined as a chronic inflammation of the middle ear and mastoid cavity, which presents with recurrent ear discharges or otorrhoea through a tympanic perforation.[1] Though the WHO definition requires only two weeks of otorrhea[1], otolaryngologists tend to adapt a duration of more than 3 months of active disease.[2]

CSOM is classified into two types depending on the site of perforation.

Tubotympanic

Also called the safe or benign type, involving the anteroinferior part of the middle ear cleft and is associated with central perforation. There is no risk of serious complications.

Atticoantral

Also called the unsafe or dangerous type, involving the posterosuperior part of the middle ear cleft and is associated with attic or marginal perforation. The disease is associated with bone eroding process such as cholesteotoma or granulations and the risk of complications are high in this type of disease.[3]

Anatomy of the Ear

Anatomically ear is divided into three parts

1. External ear

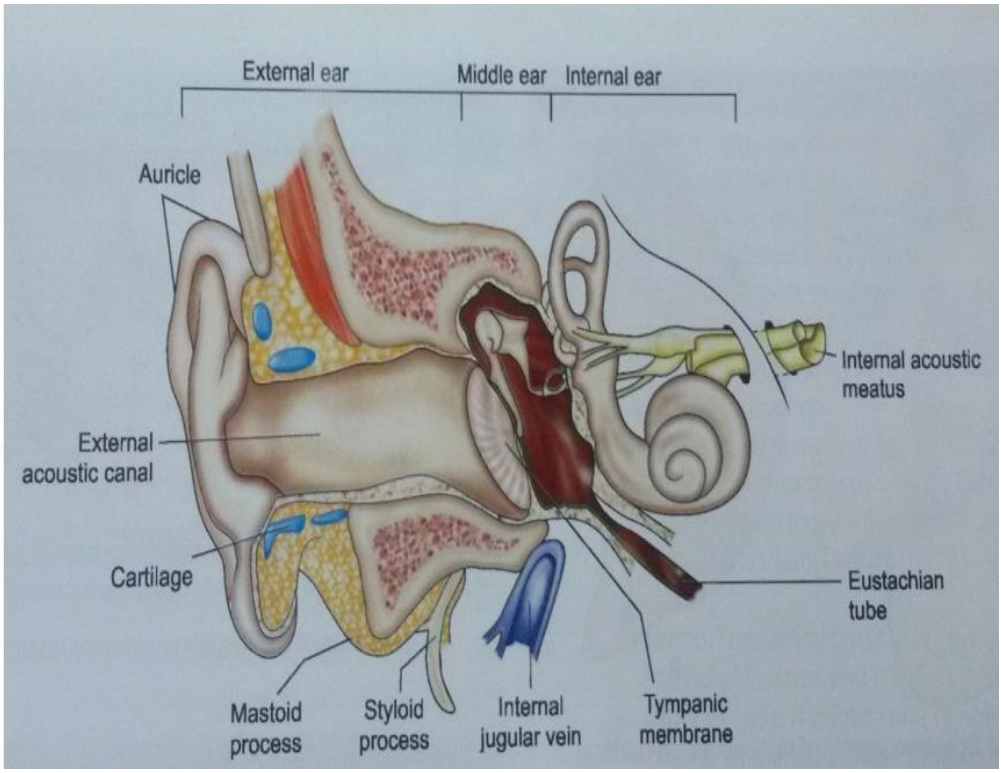
Consists of Pinna ,external auditory canal and the tympanic membrane.

The external auditory canal has two parts ,outer one third cartilaginous and inner two thirds bony.

Tympanic membrane is a greyish white membrane set obliquely which separates the external ear from the middle ear. It consists of two parts

Pars Tensa : forms most part of tympanic membrane. It is peripherally thickened to form a fibrocartilagenous ring called annulus which fits in the tympanic sulcus.

Pars Flaccida :is the superior part of tympanic membrane.



Parts of the Ear–External, Middle and Internal Ear

2. Middle Ear

Consists of the middle ear cavity with the Eustachian tube,the aditus ,the mastoid antrum and the air cells of the mastoid.

The middle ear cavity lies between the tympanic membrane laterally and the promontory formed by the basal turn of the cochlea medially and is divide into i) mesotympanum(lying opposite the pars tensa), ii) epitympanum or the attic(lying above the pars tensa) ,iii)hypotympanum(lying above the level of pars tensa).

3. Inner Ear

The inner ear or the labyrinth is an important organ for hearing and balance. It comprises the cochlea, vestibule, and semicircular canals. Vestibulocochlear nerves connect the inner ear with the brain.

Epidemiology

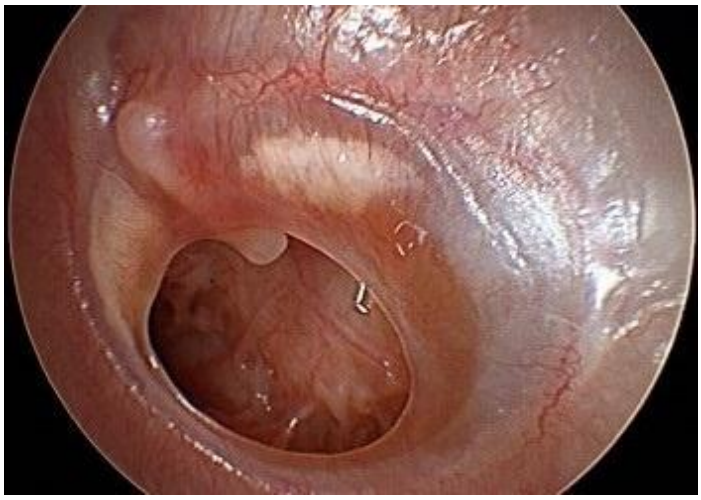
The prevalence of CSOM varies between racial and socioeconomic groups. Some of the risk factors identified include history of recurrent acute suppurative otitis media, adverse socioecomic status, overcrowded and limited access to medical care.[4] Worldwide, there are between 65-330 million people affected, of whom 60% presents with significant hearing loss. This burden falls disproportionately on children in developing countries.[11]

WHO has categorized prevalence rates of 1-2% as low and 3-6% as high prevalence group.

Country prevalence rates by WHO regional classification has categorized India as high prevalence group. (prevalence rate > 4%).[1] Incidence of CSOM in India is 46/1000(rural population)and 16/1000(urban areas) [23]. It is one of the most common causes of hearing impairment in India because of its prevalence in poor socio economic class.[24]

CSOM produces mild to moderate conductive hearing loss in more than 50% of the cases.[1]. This is due to the disruption of ear drum and ossicles which causes conductive hearing loss or from hair cell damage with the bacterial infection which has gone into the middle ear (sensory hearing loss) or both (mixed hearing loss).[1]

TUBOTYMPANIC TYPE OF CSOM



Tubotympanic Type of CSOM

In this, the disease is confined to the mucosa of Eustachian tube and anteroinferior part of middle ear. It is called safe or benign type of disease as there is no danger to the life of patient.

Predisposing factors

It is a sequelae of acute suppurative otitis media (ASOM) which has not been treated adequately.

Occasionally, a traumatic perforation may get infected leading to CSOM.

Ascending infections via Eustachian tube, infections from tonsils, adenoids and infected sinuses may be responsible for persistent or recurring otorrhoea.[3]

Abnormal Eustachian tube function is a predisposing factor seen in children with cleft palate and Down's syndrome.[4]. Other factors include allergy, malnutrion, hypogammaglobulinemia, and unhygienic personal habits like bathing and swimming in dirty water.

Viral infection would affect the mucosa of the middle ear making it less resistant to the organisms that are normally present in the middle ear ,allowing bacterial overgrowth.[2]

CSOM is often associated with poor mastoid pneumatisation. Though mastoid pneumatisation begins in the latter half of embryonic development, the greater part of this takes place in the first five years of life. Poor mastoid pneumatisation are due to

1. Infection in infancy or early childhood prevents normal cellular development.

2. Infection within a pueumatized cleft provokes sclerosis,with obliteration of the cells.

3. Failure of air-cell development predisposes to infection of the middle ear[4]

Pathogenesis

The pathological changes seen in tubotympanic type of CSOM are

1. Perforation of the pars tensa

2. Chronic inflammation of middle ear mucosa:

Hyperemia and glandular hypertrophy causing profuse discharge occurs when the disease is active. It may be normal when disease is quiescent or inactive.

3. Polyp: A polyp is a smooth mass of oedematous and inflamed mucosa which has protruded through a perforation and presents in the external canal.It is usually pale in contrast to pink, fleshy polp seen in atticoantral disease.

4. Tympanosclerosis: It is hyalinisation and subsequent calcification of the subepithelial connective tissue seen in the remnants of tympanic membrane ,ossicles ,tendons ,oval and round windows causing conductive hearing loss.

5. Ossicular chain: Fibrosis and adhesions occur as a result of healing process impairing the mobility of ossicular chain. [3]

CLINICAL FEATURES

Tubotympanic type

Ear discharge : may be continous or intermittent and varies in character from serous or mucoid or frankly purulent.

Hearing loss : predominantly conductive type of hearing loss. Factors influencing the degree of conductive deafness include size of the perforation, impairment of the ossicular chain and presence of middle ear pathology such as oedema and granulation tissue.

Investigation

Examination under microscope: provides information regarding the presence of granulations, edges of perforation, tympanosclerosis and adhesions.

Audiometry: gives an assessment of degree of hearing loss and its type which is usually conductive hearing loss.

Culture and sensitivity of ear discharge: to identify the pathogens and to select the appropriate antibiotic topical or systemic

Mastoid X rays usually sclerotic may be pneumatised with no evidence of bone destruction.

Complications of Tubotympanic type

Includes

Otitis externa, erosion of ossicular chain, sensory neural hearing loss, vertigo, tympanosclerosis and adhesions in the middle ear.

ATTICOANTRAL TYPE OF CSOM

Atticoantral Type of CSOM

It is also called unsafe or dangerous type of CSOM, as the disease spreads to bony walls of epitympanum, aditus, antrum and mastoid cells causing serious complications.

Predisposing factors

Cholesteotoma (skin in wrong place) keratinising squamous epithelium replaces the ciliated columnar epithelium of the middle ear cleft.

Middle ear mucosa undergoes metaplasia due to repeated infections and transforms to squamous epithelium.

It may also be caused by localised osteitis in which granulations are seen in the attic region.

Pathogenesis

The pathological changes in atticoantral disease include

- Retraction pocket in the attic due to negative intratympanic pressure
- Granulation tissue with keratin masses or flakes providing ideal medium for the growth of bacteria.
- Ossicular necrosis and Cholesterol granuloma[3]

Clinical features

Ear discharge: usually scanty and foul smelling due to bone destruction. blood stained discharge indicates presence of granulation and underlying osteitis.

Signs

Perforation: small attic perforation may be missed due to presence of small amount of crusted discharge.

Retraction pocket: an invagination of tympanic membrane is seen in the attic or posterosuperior part of pars tensa.

Cholesteotoma: pearly white flakes of cholesteotoma can be seen in the retraction pockets.

Investigations

Examination under microscope gives information about the extent of the defect, the presence of squamous epithelium, keratin debris and involvement of the ossicular chain or osteitis.

X ray Mastoids useful in the demonstration of anatomical variation and to detect bony erosion.

Culture and sensitivity of the ear discharge to select proper antibiotics.

Computed Tomography is generally the imaging modality of choice in the assessment of cholesteotoma.

Complications of Attico antral type

They are classified into intracranial and extracranial complications.

Intacranial: Mastoiditis, petrositis, facial paralysis and labrynthitis.

Extracranial: Extradural and subdural abscess, meningitis, brain abscess, lateral sinus thrombosis and otitic hydrocephalus.

Although incidence of complications is declining, these are still seen in india due to poor socio economic conditions ,lack of education and awareness of health care. WHO studies shows that India and Sub-Saharan Africa (SSA)

account for most deaths and years of life lost and DALYs(disability-adjusted life-years) from otitis media.[1]

Management of CSOM

The principal aims of management are the eradication of infection and the closure of the tympanic perforation.

Medical

Treatment with appropriate antibiotics based on antibiotic susceptibility test is effective in bacteriological cure.

Surgical

Correction of hearing loss and closure of tympanic membrane perforation requires appropriate surgical procedures.

Microbiological Profile of CSOM

A wide range of organisms, both aerobic and anaerobic may be isolated from cases of CSOM. Although the development if CSOM may follow an initial acute infection, the type of micro-organisms found in chronic discharge differ from those found in acute suppurative otitis media.[2] The predominance of Gram negative organisms indicates the source of infection is not the nasopharynx, which does not contain these organisms.[2].These organisms are likely to gain access to the middle ear from the external auditory canal through the tympanic membrane defect.[4]

VARIOUS ISOLATES IN CHRONIC SUPPURATIVE OTITIS MEDIA

Aerobic isolates

Gram positive Bacteria

Staphylococcus aureus

Coagulase Negative Staphylococcus aureus

Streptococcus pneumoniae

Gram negative bacteria

Pseudomonas aeruginosa,

Klebsiella pneumonia,

Proteus species,

Escherichia coli,

Acinetobacter species

Morganella morgagnii

Anaerobic isolates

Clostridium species,

Peptococcus species,

Peptostreptococcus species,

Bacteroides species.

Fungal isolates

Aspergillus niger,

Aspergillus fumigates,

Candida species

Though these bacteria are infrequently found in the external auditory canal ,they may cause infection of the middle ear due to trauma,inflammation or humidity and are most likely to gain access to the middle ear from the external auditory canal through the perforation[4].*Pseudomonas aeruginosa* has been particularly responsible for deep seated infections and progressive destruction of the middle ear and mastoid through its toxins and enzymes.

In a study by Rajat Prakash[6] the most common organism isolated was *Staphylococcus aureus* followed by *Pseudomonas aeruginosa* whereas in a study by V.K.Poorey, and Arati lyer, the commonest isolate was *Pseudomonas aeruginosa* followed by Klebsiella species[7]. Many of the previous studies showed Pseudomonas to be the most predominant isolate.[25,26,27,28,29].

Microbiological characteristics of Pseudomonas species

Pseudomonas are ubiquitous microorganisms ,found in the environments such as water, soil, plants, humans, animals, sewage and hospilats. In humans they are opportunistic pathogens and has great propensity to grow in a variety of environments with minimal nutritional components.[16]

It is resistant to the common antiseptics and disinfectants,such as quaternary ammonium compounds, chloroxylenol and hexachlorophene and may even grow profusely in bottles of antiseptic lotions .

Historical Perspective

Until the first half of twentieth century ,the description were inadequate in terms of phenotypic characterisation. Work performed at the university of California,Berkeley proposed the system of classification of pseudomonas species based on phenotypic characteristics.(Stainer et al.1966).Later this was followed by DNA-DNA hybridisation studies that constituted a group that was not phylogenitically uniform.

A clear demonstration of the phylogenetic heterogenicity was eventually achieved by ribosomal RNA(rRNA)-DNA hybridisation as an analytical criterion(Palleroni et al.1973).[17]

Classification

Palleroni separated the pseudomonas into five ribosomal RNA homology groups based on rRNA-DNA homology studies whereas Gilardi separated pseudomonas into seven major groups based on phenotypic charecteristics.[18]

Phenotypic and Genotypic classification of Pseudomonas is as given below.

RNA Group I

Fluorescent Group : P.aeruginosa, P. Fluorescens, P. putida.

Stutzeri group : P.stutzeri,P.mendocina,CDC Group Vb-3.

Alkaligenes Group : P.alcaligenes,P.pseudoalcaligenes ,

Pseudomonas species group 1 .

RNA Group II

Pseudomallei Group : Burkholderia mallei,Burkholderia pseudomallei

Burkholderia cepacia complex,

Burkholderia gladioli,Pandoraea species,

Ralstonia species,Cupriavidus species.

RNA Group III

Weak Oxidiser Group : Comamonas acidovorans,C.terrigena,C.testosteroni,

Acidovorax delafieldii,A.facilis,A.temperans,

Lautropia mirabilis, CDC WO-1.

RNA Group IV

Diminuta Group : Brevundimonas diminuta,B.vesicularis.

RNA Group V : Stenotrophomonas maltophila

Yellow-Pigmented Group: P.luteola,P.orizohabitans,

Sphingomonas.paucimobilis.

H2S-Positive Group: Shewanella putrifaciens,Shewanella algae.

Halophilic Group: Alishewanella fetalis,Halomonas venusta,

CDC halophilic nonfermenter group 1

Morphology and Cultural Characteristics

- *Pseudomonas aeruginosa* are straight gram negative rod measuring 0.5 to 0.8 µm by 1.5 to 3.0 µm ,that are strict aerobes and motile by means of polar flagellum.They utilise glucose and other carbohydrates oxidatively and are cytochrome oxidase positive.
- *P.aeruginosa* grow well on ordinary media ,and produce a characteristic fruity grape like odour.
- On MacConkey agar they grow as non lactose fermenting colonies with green pigmentation or metallic sheen.
- On Blood agar the colonies appear as large colonies with metallic sheen, mucoid, rough,or pigmented and often beta haemolytic.
- The colonies of *Pseudomonas aeruginosa* are usually of two types on most solid media.

- One is large and smooth with flat,edges and elevated centres and the other is smooth, rough and convex.Clinical isolates are generally of large type ,while the small type is commonly obtained from natural sources.Variation of the large type to small is observed frequently but the reverse is rare.
- A third type of mucoid colony can be obtained from respiratory and urinary tract secretions.The mucoid morphotype is due to the production of polysaccharide ,alginate that surrounds the cell.
- Additional colony types can be observed and Jessen(1965) states that at least five types may be distinguished but the type of colony cannot be correlated with other properties of the strain.[17]
- Circular,smooth colonies.

  Irregular,contoured colonies

  Dry,flat colonies

  Mucoid colonies

  Rugose colonies

Biochemically they are nonfermenter, oxidase positive which can grow optimally at 37° C and can be distinguished from the others in the group by its ability to grow at 42° C.They exhibit the characteristic pigment production of pyocyanin and pyoverdin.

Key Characteristics of Fluorescent group

TEST	***P.aeruginosa***	***P.fluorescens***	***P.putida***
Pyoverdin	+	+	+
Pyocyanin	+	_	_
Acetamide	V	_	_
Growth at 42 C	+	_	_
No3 reduction	V(74)	V(19)	_
Gelatin hydrolysis	V(46)	+	_

+ , 90% or more strains positive

- , 90%or more srains negative

V, 11-89%of strains positive.

PIGMENTATION

Fluorescent (pyoverdin)Pigment

The Fluorescent group is characterised by production of water soluble pyoverdin pigment that fluoresces white to blue-green under ultraviolet light.

Pyocyanin pigment

Though all the three members of fluorescent group produce pyoverdin, *P.aeruginosa* is the only species that produces the distinctive blue pyocyanin pigment.

Other water soluble pigments include pyorubin and pyomelanin which imparts red and brown colour respectively.

Pigment production can be enhanced by using Tech and Flo media which contains special peptones and increased concentration of magnesium and sulphate ions.

Other methods to enhance pigment production is by growing the organisms in gelatin, potato or milk containing media.

Virulence factor

Pseudomonas aeruginosa is the most common opportunistic pathogen of all pseudomonas species .It produces several substances that enhance the colonization and infection of host tissue.

Virulence Factors of *Pseudomonas aeruginosa*

Virulence Factor	Biological activity
Alginate	Capsular polysaccharide helps to adhere to the epithelial surface of lungs and forms biofilms.
Pili	Surface appendages that cause adherence to GM-1 ganglioside receptors of epithelial cell surfaces.
Neuraminidase	Removes sialic acid residues from GM -1 ganglioside receptors,facilitation binding of pili.
Lipopolysaccharide	Produces endotoxin which causes sepsis syndrome.
Exotoxin A	Tissue destruction,inhibition of protein synthesis.
Enterotoxin	Interrupts normal gastrointestinal activity,leading to diarrhoea.
Exoenzyme S	Inhibits protein synthesis.
Phosholipase C	Destroys cytoplasmic membrane,destroys pulmonary surfactant,inactivates opsonins.
Elastase	Cleaves immunoglobulins and complement,distrups neutrophil activity.
Leukocidin	Inhibits neutrophil and lymphocyte function.
Pyocyanins	Suppress other bacteria and distrups respiratory ciliary activity,cause oxidative damage to tissues particularly lung.

Of all the extracellular enzymes produced by *P.aeruginosa*, exotoxin A is the most toxic causing inhibition of peptide chain elongation and protein synthesis.[17]

Resistance to antibiotics

P.aeruginosa is notorious for its resistance to antibiotics with more than 50 resistant genes.

The general resistance is due to combination of factors such as low permeability of the cell wall, genetic capacity to express a wide repertoire of resistance mechanisms, resistance through chromosomal mutations and acquiring resistant gene from other organisms via plasmids, transposons and bacteriophages.

The three major mechanisms by which the organisms resist the action of antimicrobial agents include

1. Restricted uptake and efflux
2. Drug inactivation and
3. Mutational changes in target enzymes.[13]

Mechanisms of Resistance

Intrinsic Resistance

Intrinsic Resistance is mediated through multiple efflux pumps,resulting in expulsion of betalactams, chloramphenicol, fluroquinolones, macrolides , sulphonamides ,tetracycline and trimethoprim.

Extrinsic Resistance

Various antibiotics that overcome the intrinsic resistance include, extended spectrum penicillins (piperacillin, ticarcillin),certain third and fourth generation cephalosporins,(ceftazidime and cefipime),carbapenem (imipemem and meropenem),monobactams(aztreonam),fluoroquinolones(ciprofloxacin and levofloxacin),aminoglycosides (gentamicin, tobramycin, and amikacin) and colistin.

But mutational resistance can develop for these antibiotics.

Penetration of antibiotics through cell wall

The outer membrane of *P. aeruginosa* presents a significant barrier to the penetration of antibiotics, restricting the rate of penetration of small hydrophilic molecules and excluding larger molecules.

Hydrophilic antibiotics such as ß-lactams and quinolones can only cross the outer membrane by passing through the aqueous channels provided by porin proteins. These porin proteins are barrel shaped associated as trimers. OprD is a specialized porin which has a specific role in the uptake of positively charged amino acids.

Impermeability Mutations is important in carbapenem resistance due to the loss of OprD porin, a protein that forms a narrow transmembrane channel permeable to carnapenems but not betalactams.

Loss of oprD is frequently associated with resistance to imipenem, which requires this porin to cross the outer membrane. Interestingly, meropenem is not affected by loss of oprD, indicating that the carbapenems

have crossed the outer membrane by different channels.[13]. Resistance to aminoglycosides and colistin has due to overexpression of an outer membrane protein, oprH, which protects the LPS from binding the antibiotics has been observed in laboratory strains of P.aeruginosa but less frequently in clinical isolates.[30]

Efflux Pumps

Efflux pumps play an important role in multi drug resistant comprising of three protein components, an energy-dependent pump located in the cytoplasmic membrane, an outer membrane porin and a linker protein which couples the two membrane components together.[31]

The different antibiotic efflux systems that have been described in P. aeruginosa are mexAB-oprM, mexXY-oprM, mexCD-oprJ and mexEF-oprN.[32]

mexAB-oprM is responsible for extrusion of ß-lactams, quinolones and a range of disinfectants. mexXY-oprM extrudes aminoglycosides mexEF-oprN extrudes carbapenems and quinolones.

The genes are present in all strains but they are not expressed at high levels. However, increased expression result from mutation of the regulatory genes such as mexR, which controls expression of the mexAB-oprM genes.[33].

Mutation or upregulation of mexR repressor gene results in efflux pump overproduction and increase in the minimum inhibitory concentration(MIC) of multiple antibiotics but not imipenem.

DRUG INACTIVATION

Inactivation of aminoglycosides occurs through production of enzymes which transfer acetyl, phosphate or adenylyl groups to amino and hydroxyl substituents on the antibiotics. The modifying enzymes are plasmid mediated, consequently spontaneous mutations in cells during antibiotic treatment does not lead to overexpression of the enzymes, as seen with the chromosomal ß-lactamases.

CHANGES IN TARGET ENZYMES

In *P. aeruginosa* changes in target enzymes is most commonly encountered with the quinolones through mutation in the gyrA gene encoding the A subunit of the target enzyme, DNA gyrase.[34]

METHODS TO DETECT ANTIBIOTIC RESISTANCE MECHANISMS

Phenotypic methods

Extended Spectrum Betalactamase

Represent betalactamases that hydrolyze extended-spectrum cephalosporins (cefotaxime, ceftriaxone, ceftazidime), monobactams (aztreonam) and are not affected by betalactamase and carbapenemase inhibitors. [35]

Confirmatory methods

- Double disk diffusion synergy test[39]
- Three dimensional test
- Inhibitor potentiated disc diffusion test[39]

- ESBL E-strip method
- ESBL agar medium

Amp C betalactamase detection

Isolates showing reduced susceptibility to cefoxitin were considered as screen positive and selected for detection of Amp C betalactamases.

Confirmatory methods

- Amp C disc test
- Modified three dimensional test[40]
- Amp C betalactamase E test[40]

Carbapenemase detection

A strain that produces carbapenemases presents at least 21 mm diameter to meropenem, imipenem or ertapenem is considered to be positive for Carbapenemase production. [36]

- Confirmatory methods
- Modified Hodge test
- Imipenem- EDTA combined disc test
- Imipenem EDTA double disc synergy test
- MBL E-test
- CARBA NP test

MATERIALS AND METHODS

This cross sectional study was conducted in the Institute of Microbiology, in association with Upgraded institute of Otorhinolaryngology, Madras Medical College ,Rajiv Gandhi Government General Hospital, Chennai.

Study period

The study period is for a period of 1 year from October 2014 to September 2015.

Study Population

100 patients with clinical diagnosis of CSOM attending ENT department of RGGGH who satisfied the inclusion criteria were enrolled for the study.

Ethical consideration

Approval was obtained from the Institutional ethics committee before the commencement of the study. Informed consent was obtained from all the patients who participated in the study.

Statistical analysis

Statistical analysis were carried out using Statistical Package for Social Sciences(SPSS).The proportional data of this cross sectional study were tested using One sample Z-test.

Study population

A total of 100 patients with clinical diagnosis of CSOM from the Upgraded Institute of Otorhinolaryngology ,RGGGH ,Chennai were include in the study.

Inclusion criteria

- Patients with clinical diagnosis of CSOM
- Patients older than 13 years.
- Patients who were not on antibiotic (both systemic and topical) treatment for minimum of 24 hours prior to sample collection.

Exclusion criteria

- Patients less than 13 years.
- Patients with acute otitis media.
- Patients not willing to participate in the study.

Sample collection

The ear discharge was collected using sterile swabs under aseptic precautions with the aid of an aural speculum, prior to the instillation of any topical medication. Using sterile swabs .A minimum of three samples were taken. First swab used for direct Gram stain and KOH mount,Second swab for bacterial culture and the third swab for fungal culture based on the KOH mount.

Specimen processing

- Direct Gram stain :

  Shows possible pathogens present in sample.

- KOH mount:

  Detection of fungal elements.

Interpretation of bacterial culture

The swab on reaching the laboratory was inoculated on the following culture media

- Nutrient agar plate
- Mac Conkey agar plate
- 5% Sheep Blood agar plate

After overnight incubation at 37°C aerobically, the plates were examined for growth and culture characteristics were identified.

The isolates were identified by Grams stain morphology, motility, culture characteristics and biochemical reactions by the standard techniques.

The isolated colonies depending on the Gram reaction were subjected to following biochemical tests for identification.

If Gram negative bacilli was seen ,the colonies were subjected to the following tests and biochemical reactions using standard micobiological techniques.

1. Catalase test
2. Oxidase test

3. Nitrate reduction test

4. Hugh-Leifson’s Oxidation Fermentation test

5. Indole test

6. Methyl red test

7. Voges Proskauer test

8. Simmon’s Citrate utilization test

9. Christensen’s Urease test

10. Mannitol motility

11. Triple sugar iron agar

12. 1% Sugar fermentation tests Glucose,Sucrose,Maltose,Mannitol.

13. Lysine decarboxylase,Ornithine decarboxylase and Arginine dihydrolase test.

Identification of *Pseudomonas species* was based on the following culture characteristics and biochemical reactions.

On Nutrient agar- iridescent colonies with metallic sheen are seen.

On MacConkey agar - forms non-lactose fermenting colonies .

On Blood agar - many strains were hemolytic on blood agar. Some of them were non hemolytic.

Isolates that are GramNegative bacilli,catalase positive,oxidase positive and motile by hanging drop were identified as *Pseudomonas species*.

Isolates were identified as *Pseudomonas aeruginosa* by the following characteristics

1. Gram Negative rod
2. Oxidase Positive
3. Fruity grape –like odour
4. Growth at 42°C :P.aeruginosa is distinct from the rest of the clinically relevant fluorescent pseudomonas in its ability to grow at 42°C.
5. Nitrate reduction and gas from nitrate
6. Arginine dihyhrolase test :Positive
7. Gelatin Hydrolysis :Positive

Acinetobacter speciation was done by the following additional tests

1. Presence of growth at 44°C
2. Presence of ß hemolysis
3. 10% OF lactose utilization test
4. Malonate utilization.

Isolates that are Gram Positive cocci were subjected to the following tests and biochemical reactions using standard techniques.

1. Catalase test
2. Coagulase test -Slide and Tube coagulase

3. Modified Oxidase test

4. Urease Test

5. Mannitol fermentation test

6. Phenolphthalein phosphatase test

7. Gelatin Liquifaction test

8. Bacitracin susceptibility test using 0.04U disk.

If the Gram positive cocci in clusters that were catalase positive and coagulase negative it was identified as Coagulase negative Staphylococci and the following additional tests were done for speciation of CONS.

1. Carbohydrate fermentation tests using Lactose, Mannitol, Mannose, Xylose and Trehalose

2. Nitrate reduction test

3. Ornitine decarboxylase test

4. Differential disc diffusion test with Novobiocin (5µg) and Polymyxin 300 units.

ANTIBIOTIC SUSEPTIBILITY TESTING

Isolates were subjected for antibiotic susceptibility testing by employing Kirby-Bauer standard disc diffusion method on Muller- Hinton agar according to CLSI guidelines (M100-S24)

Antimicrobial susceptibility testing by Kirby-Bauer Disc diffusion method

1. With a sterile bacteriological wire loop 3-5 well isolated identical colonies were picked up and inoculated in 5ml of peptone water, incubated at 37°C for 3-5 hrs to attain 0.5 McFarland's turbidity.
2. A sterile cotton swab was dipped into it and pressed firmly against the wall of the test tube to remove excess broth from the swab.
3. Dried surface of Mueller Hinton agar plate was swabbed in three directions 60° each time to ensure an even and complete distribution of the inoculums over the entire plate.
4. Inoculated plate was allowed to dry for 3 to 5 minutes with the lid in place before adding the antibiotic discs.
5. The antimicrobial discs were placed on the surface of the agar using forceps. Not more than 6 discs were placed in the plate.
6. After overnight incubation at 37°C, the diameter of zone of inhibition was measured in mm.

Interpretation of Zone of inhibition diameters were done according to CLSI guidelines.

ATCC control strains:

- *Staphylococcus aureus*–ATCC 25923
- *Escherichia coli*-ATCC 25922
- *Pseudomonas aeruginosa*-ATCC 27853
- *Klebsiella pneumoniae* (ESBL)-ATCC 700603

The panel of drugs used for antimicrobial susceptibility test of Gram Positive organisms were as follows

Antibiotic	**Disc content**	**Zone of Inhibition in mm**		
		Sensitive	**Intermediate**	**Resistant**
Penicillin	10 units	≥ 29	-	≤28
Erythromicin	30µg	≥23	14-22	≤13
Ciprofloxacin	5µg	≥21	16-20	≤15
Amikacin	30µg	≥17	15-16	≤14
Cotrimoxazole	1.25/23.75µg	≥16	11-15	≤10
Chloramphenicol	30µg	≥18	13-17	≤12

The panel of drugs used for antimicrobial susceptibility test of Gram Negative organisms were as follows

Antibiotic	Disc content	Gram negative bacilli	Diameter of Zone of inhibition in mm.		
			Sensitive	Inter mediate	Resistant
Amikacin	30µg	Enterobacteriaceae &Non-fermenters	≥ 17	15-16	≤ 14
Ciprofloxacin	5 µg	Enterobacteriaceae &Non-fermenters	≥21	18-20	≤17
Gentamicin	10µg	Enterobacteriaceae &Non-fermenters	≥15	13-14	≤12
Cotrimoxazole	1.25/ 23.75µg	Enterobacteriaceae &Non-fermenters	≥16	11-15	≤10
Piperacillin-Tazobactam	100µg /10µg	Enterobacteriaceae &Non-fermenters	≥21	18-20	≤17
Cefotaxime	30µg	Enterobacteriaceae	≥26	23-25	≤22
		Acinetobacter	≥23	15-22	≤14
Ceftazidime	30µg	Enterobacteriaceae	≥21	18-20	≤17
		P.aeruginosa& Acinetobacter sp.	≥18	15-17	≤14
Imipenem	10µg	Enterobacteriaceae	≥23	20-22	≤19
		P.aeruginosa	≥19	16-18	≤15

All the isolates of Pseudomonas aeruginosa were screened for ESBL, AmpC and MBL production by the following methods. The screen test positive isolates were subjected to respective confirmatory tests using appropriate antibiotic discs.

Extended spectrum ß-lactamase(ESBL) detection method.

Pseudomonas aeruginosa showing reduced zone of inhibition around ceftazidime(30µg) with reference to CLSI 2014 AST criteria were considered to be ESBL producers.

Using sterile loop, four or five colonies of similar morphology were picked up, inoculated to 5ml peptone water and incubated at 37°C for 4-6hrs until turbidity matches with McFarland 0.5 turbidity standard ($1.5x10^8$cfu/ml). Lawn culture was done on Mueller-Hinton agar plates and Ceftazidime and Ceftazidime-clavulanic acid discs are placed 50mm apart from centre to centre.

Interpretation

Zone of inhibition was measured around the discs with the help of measuring scale. An increase in 5 mm in zone of inhibition in a disc containing clavulanic acid compared to the drug alone is considered as an ESBL producer.

AmpC ß lactamases detection methods

Screening for AmpC ß-lactamase

Pseudomonas isolates were screened for AmpC ß-lactamase by using cefoxitin (30µg).Isolates showing reduced susceptibility to cefoxitin (30µg)

with zone diameter less than 18mm were considered as positive and selected for the detection of AmpC ß-lactamases by Amp C disc test.[37]

Amp C disc test

- A lawn culture of ATCC E.coli 25922 was prepared on MHA plate.
- Sterile discs of 6mm size was moistened with sterile saline and inoculated with several colonies of test organism.
- The inoculated disc was then placed beside a cefoxitin disc (almost touching on the inoculated plate.The plates were incubated overnight at 35°C.
- Flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disc were considered as AmpC positive isolate.
- A negative test had an undistorted zone.

Metallo-ß-lactamase (MBL) detection method

Screening method for MBL

Pseudomonas isolates were screened for Metallo-ß-lactamase production by using imipenem (10µg).

Isolates with zone of inhibition less than 15mm were considered as MBL producers.[38].

DETECTION OF METHICILLIN RESISTANCE IN STAPHYLOCOCCUS AUREUS

Isolates of Staphylococcus aureus were screened for MRSA by standard disc diffusion using cefoxitin(30µg).Isolates with zone of inhibition less than 21mm were considered as Methicillin resistant Satphylococcus aureus.

FUNGAL CULTURE

KOH Mount

Direct microscopic examination of the ear discharge was done in 10% KOH. for the presence of epithelial cells ,pus cells, budding yeast cells ,fungal hyphae and spores.

One large drop of 10% KOH was placed on a clean grease free slide mounted with a small quantity of specimen and is mixed well.A sterile cover slip is placed over the drop and left at room temperature for 10 mts for the digestion of the debris.The mount is examined under low and high power for the presence of epithelial cells ,pus cells,budding yeast cells ,fungal hyphae and spores.

INTERPRETATION OF FUNGAL CULTURE

Based on the KOH mount, the ear discharge was also inoculated onto 2 slopes of Sabourad's dextrose agar of pH 5.6 with antibiotics like gentamicin to inhibit bacterial growth and incubated at 25°C and 37°C for 4-6 weeks.The slopes were examined daily during first week and twice a week for the next three weeks.failure of growth even after six weeks was considered as negative for fungal growth and were discarded.

The significance of fungal culture ,in case of commensal or opportunistic fungi being isolated can be established by the following features.

- Isolation of same fungal isolate from all the culture tubes.
- Direct microscopic confirmation of the fungal elements.
- Repeated isolation of the isolate from multiple specimens.

Tubes showing positive cultures were examined for macroscopic and microscopic appearance of the colonies. Any visible growth on either of the slants were examined for Rate of growth, texure, surface pigmentation, pigmentation on the reverse, presence of diffusible pigment.

Microscopic examination was done by Lactophenol cotton blue(LPCB) mount

A drop of LPCB was placed on a grease free slide and a small amount of fungal colony midway between the colony edge and centre was placed on the LPCB drop. The growth was teased so as to have a thin spread out and a coverslip is gently dropped to the edge of the mounting fluid to avoid air bubbles. The mount is examined under the microscope.

Identification is based on the following characteristics

- Nature of hyphae (such as septate or aseptate,hyaline or phaeoid,narrow or wide)
- Conidiogenesis (origin,arrangement)
- Conidia (septate,Pigmented or hyaline,shape and conidial wall)

RESULTS

This cross sectional study was conducted in the Institute of Microbiology, Madras Medical College in association with the Upgraded Institute of Otorhinolaryngology, at the Rajiv Gandhi Government General Hospital, Chennai .

A total of 100 patients with CSOM who satisfied the inclusion criteria were included in the study.Out of 100 patients 93 were culture positive while 7 samples showed no growth.

TABLE 1: ANALYSIS OF AGE DISTRIBUTION IN CSOM

Age Distribution Vs Culture Results	Number of Cases	Positive Cases	%	NO Growth	%
11-20 Years	8	5	5.38	3	42.86
21-30 Years	26	24	25.81	2	28.57
31-40 years	24	24	25.81	0	0.00
41-50 Years	16	15	16.13	1	14.29
51-60 Years	16	15	16.13	1	14.29
61-70 Years	9	9	9.68	0	0.00
71-80 Years	1	1	1.08	0	0.00
Total	100	93	100	**7**	100

Table 1 shows the Age wise distribution of the morphotypes in CSOM. Maximum number of patients were in the second decade of life.

FIG. 1: ANALYSIS OF AGE DISTRIBUTION IN CSOM

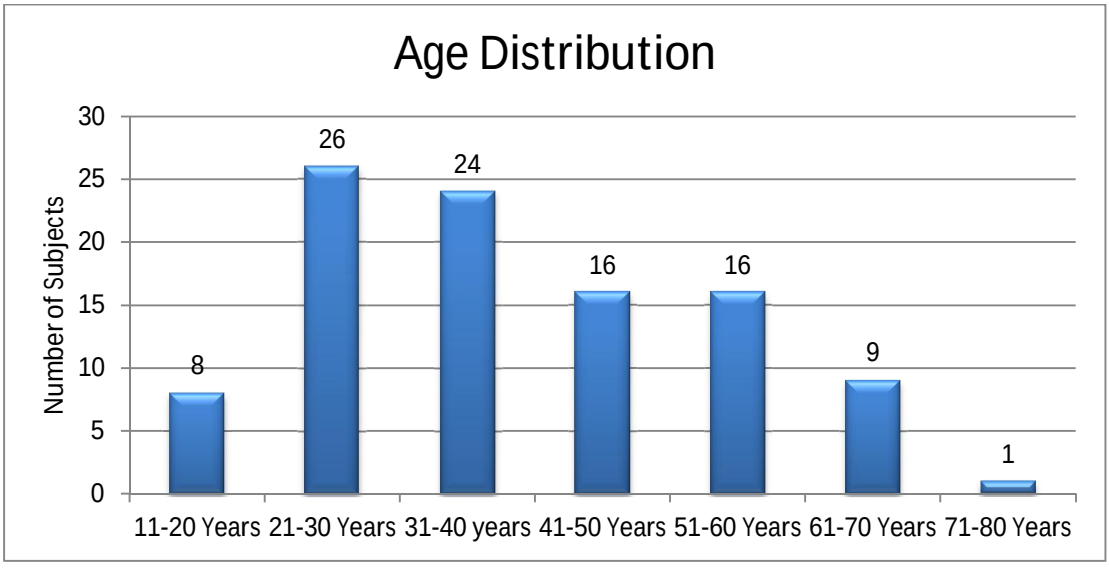


TABLE 2: ANALYSIS OF GENDER DISTRIBUTION

Gender Distribution	Frequency	Percentage
Male	57	57
Female	43	43
Total	100	100
P value One Sample Z-Test		0.321

The gender distribution were studied and was found that Males outnumbered females in the ratio of 1.3 : 1.

FIG.2: ANALYSIS OF GENDER DISTRIBUTION

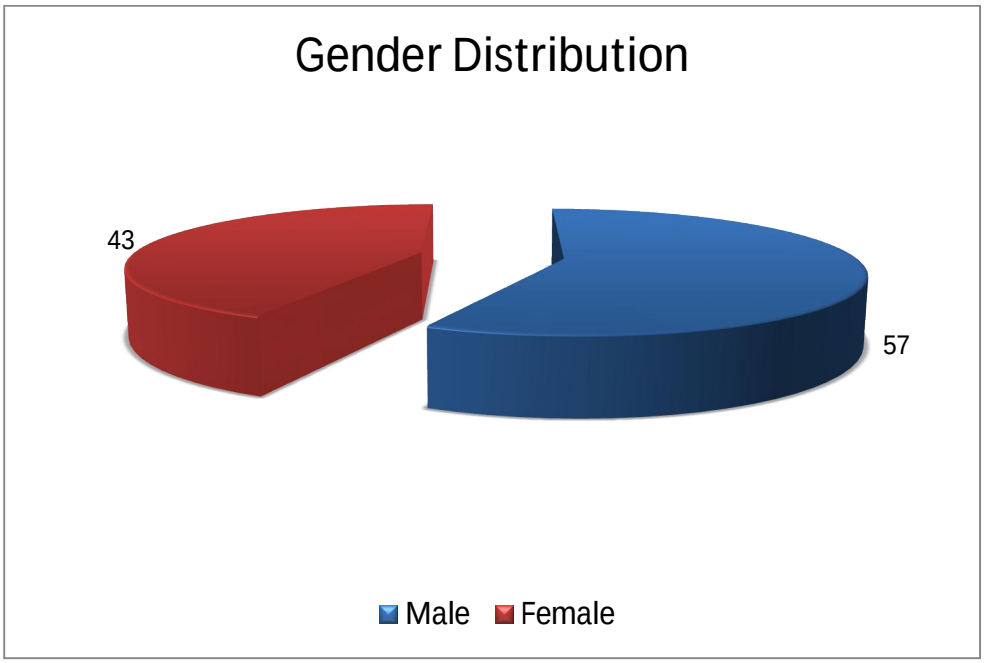


TABLE : 3 DISTRIBUTION OF RISK FACTORS ASSOCIATED WITH CSOM

Associated Features	Number
URTI	42
Allergy	18
Tonsillitis	15
DNS	43

FIG. 3: DISTRIBUTION OF RISK FACTORS ASSOCIATED WITH CSOM

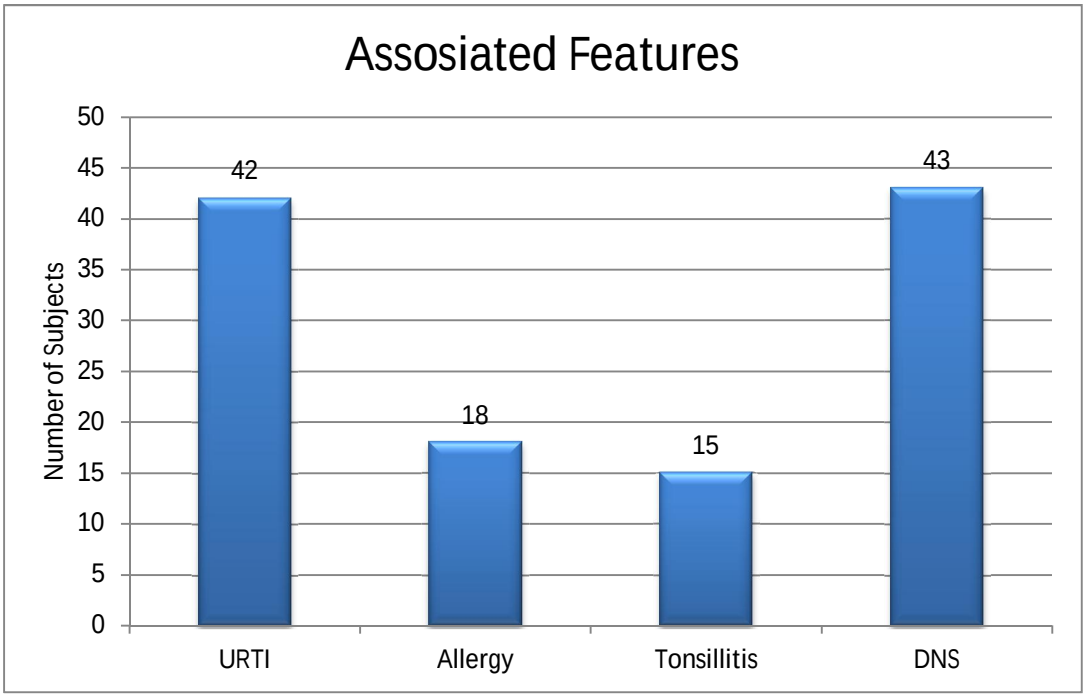


TABLE 4 : DISTRIBUTION OF UNILATERAL AND BILATERAL CSOM

	Number	%
Unilateral CSOM	95	95%
Bilateral CSOM	5	5%

Among the 100 patients included in the study, 95 patients has unilateral ear discharge and 5 had bilateral ear discharge .

FIG. 4: DISTRIBUTION OF UNILATERAL AND BILATERAL CSOM

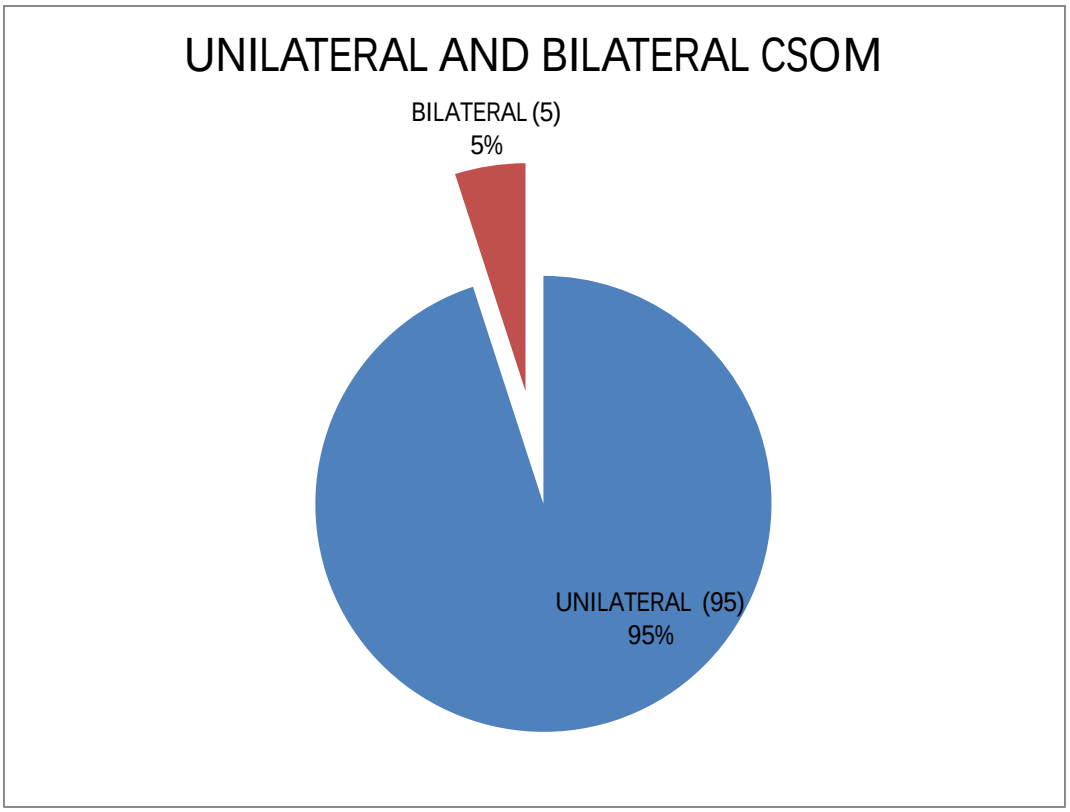


TABLE 5: DISTRIBUTION ACCORDING TO THE TYPE OF CSOM

Type of CSOM	Number	%
Tubotympanic CSOM	88	88.00
Atticoantral CSOM	12	12.00
Total	100	100
P value One Sample Z-Test		0.0018

FIG. 5: DISTRIBUTION ACCORDING TO THE TYPE OF CSOM

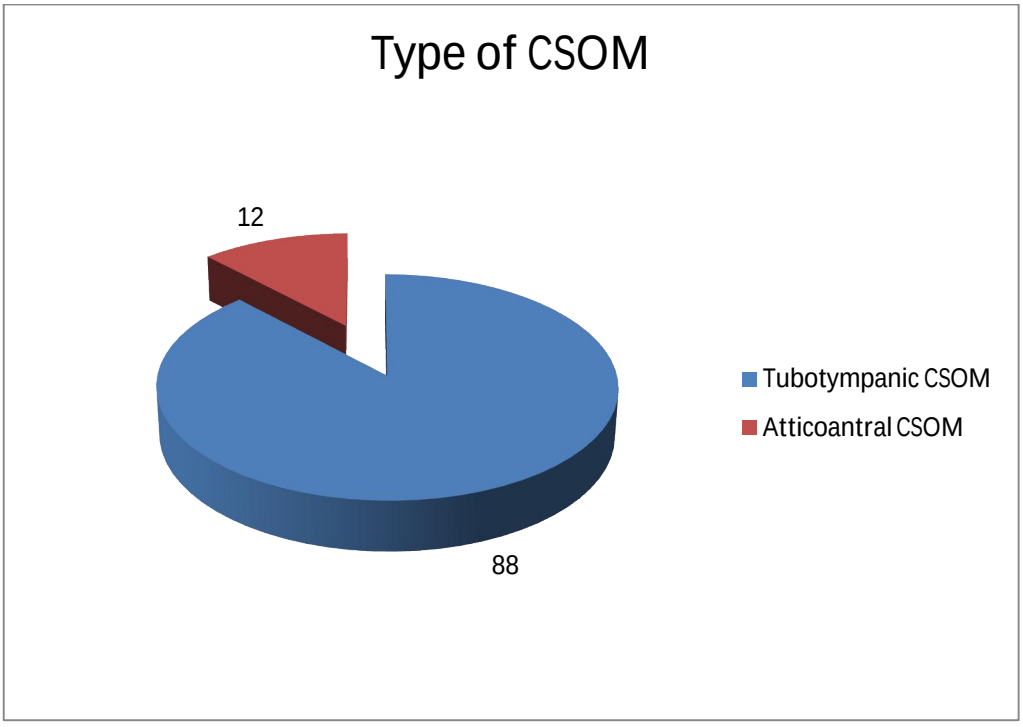


TABLE 6 : ANALYSIS OF CULTURE RESULTS

Culture results	Number	Percentage
Positive	93	93%
Negative	7	7%

FIG. 6: ANALYSIS OF CULTURE RESULTS

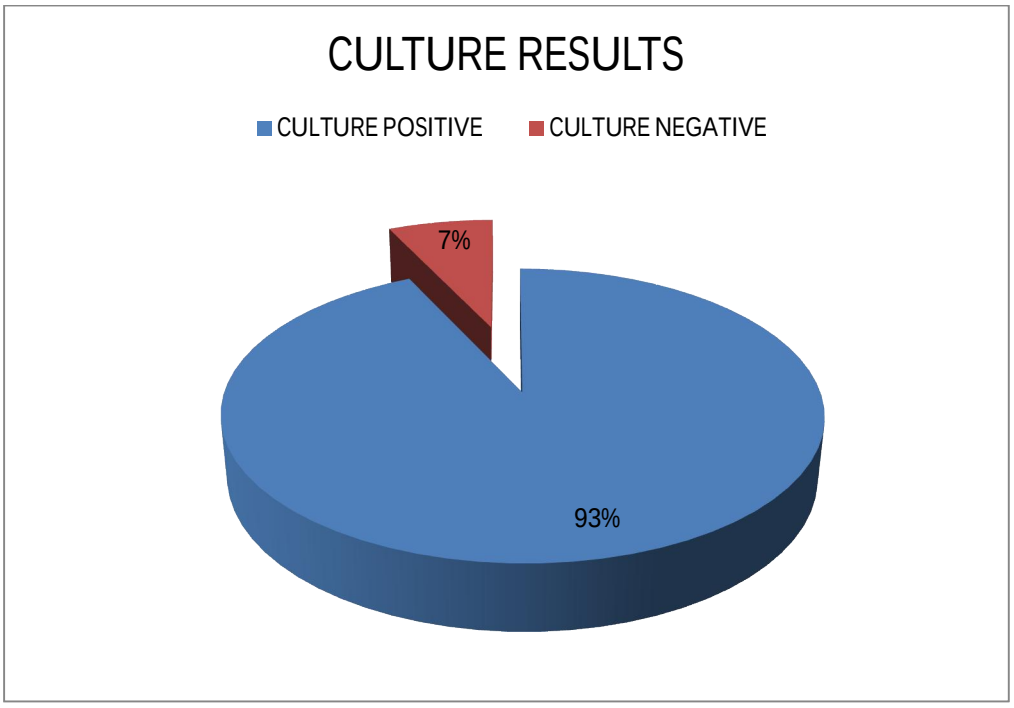


TABLE 7 : MONOMICROBIAL AND POLYMICROBIAL DISTRIBUTION IN CSOM

Age Vs microbial Isolates	Monomicrobial	%	Polymicrobial	%
11-20 Years	5	5.61%	0	0.00
21-30 Years	23	25.84%	2	28.57%
31-40 years	20	22.47%	4	57.14
41-50 Years	15	16.85%	0	0.00
51-60 Years	3	3.37%	0	0.00
61-70 Years	22	24.71%	1	14.28%
71-80 Years	1	1.12%	0	0.00
Total	89	100%	7	100%

Monomicrobial growth was seen in 89 patients, whereas polymicrobial growth was observed in 7 patients .

TABLE 8: MICROBIAL PROFILE

Organism	No.of Isolates	%
Pseudomonas aeruginosa	41	39.81
Klebsiella pneumonia	7	6.80
Escherichia coli	5	4.85
Proteus mirabilis	4	3.88
Acinetobacter baumannii	3	2.91
Staphylococcus aureus	32	31.07
Staphylococcus epidermidis	5	4.85
Aspergillus niger	4	3.88
Aspergillus fumigatus	2	1.94
Total	103	100

FIG. 7: MICROBIAL PROFILE

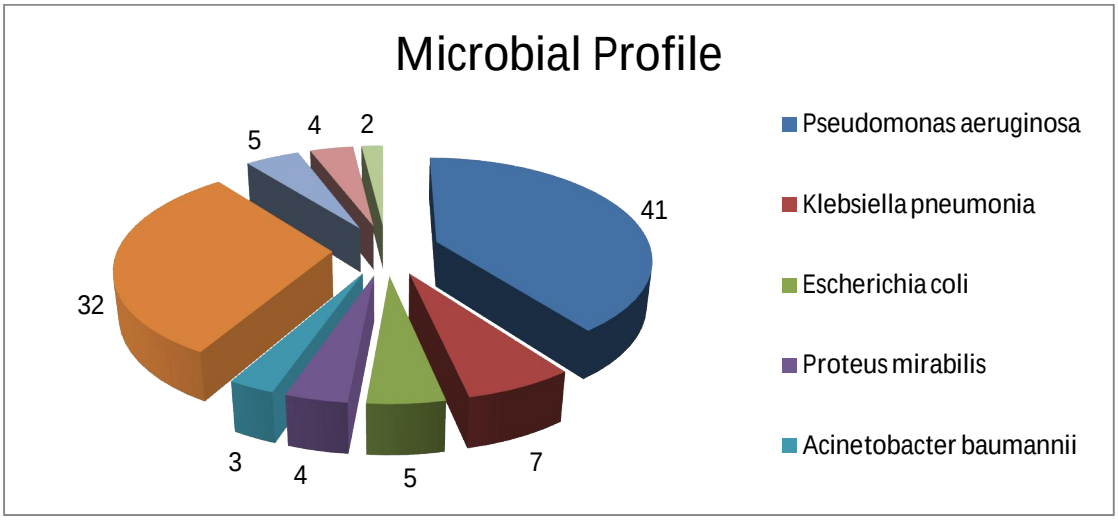


TABLE 9: DISTRIBUTION OF GRAM NEGATIVE BACTERIA IN CSOM

Gram Negative Bacteria	No.of Isolates	%
Pseudomonas aeruginosa	41	68.33
Klebsiella pneumoniae	7	11.67
Escherichia coli	5	8.33
Proteus mirabilis	4	6.67
Acinetobacter baumannii	3	5.00
Total	60	100

FIG.8. DISTRIBUTION OF GRAM NEGATIVE ISOLATES IN CSOM

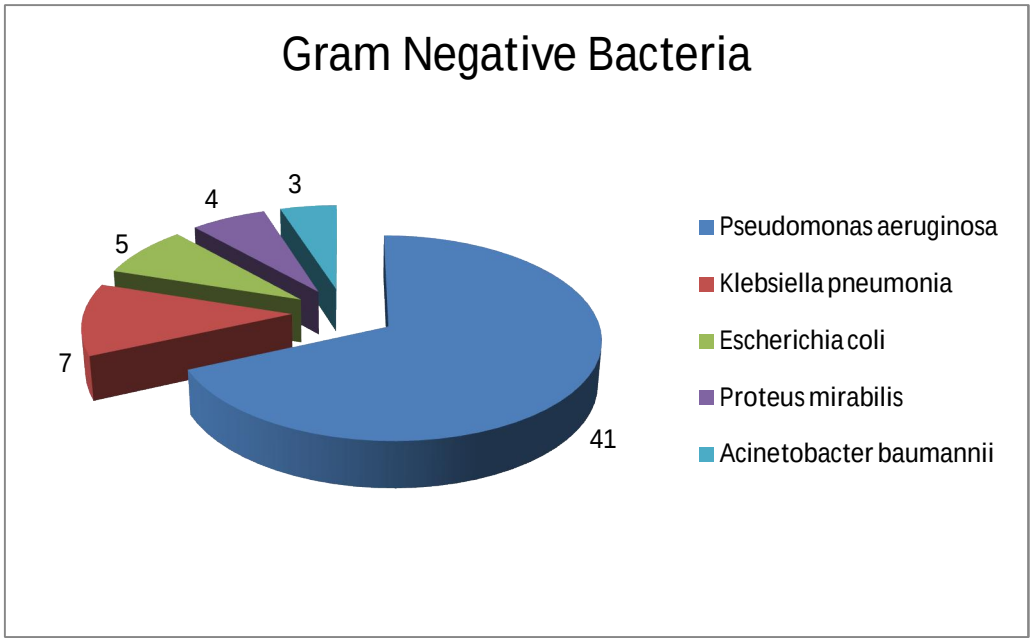


TABLE 10: ANALYSIS OF GRAM POSTIVE ISOLATES IN CSOM

Gram Positive Cocci	No.of Isolates	%
Staphylococcus aureus	**32**	**86.49**
Staphylococcus epidermidis	**5**	**13.51**
Total	**37**	**100**

FIG.9. ANALYSIS OF GRAM POSITIVE ISOLATES IN CSOM

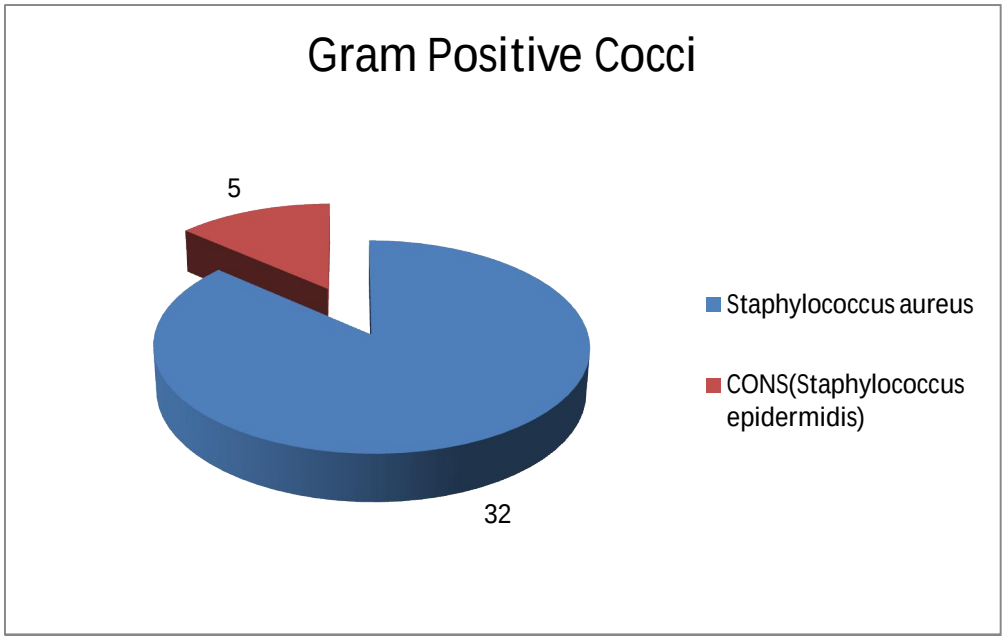


TABLE :11 LIST OF FUNGI ISOLATED IN CSOM

Fungal	No.of Isolates	%
Aspergillus niger	4	66.67
Aspergillus fumigatus	2	33.33

Fungal culture was positive in 6 patients, out of which 4 were A.fumigatus and 2 were A.niger.

TABLE 12:ISOLATES IN POLYMICROBIAL PROFILE

Orgainsms	Number of patients
S.aureus + E.coli	2
P.aeruginosa + K.pneumoniae	1
K.pneumonia+S.epidermidis	1
P.aeruginosa +S.aureus	1
S.aureus + A.baumannii	1
P.aeruginosa +A.niger	1
Total	7

FIG.10. ISOLATES ON POLYMICROBIAL PROFILE

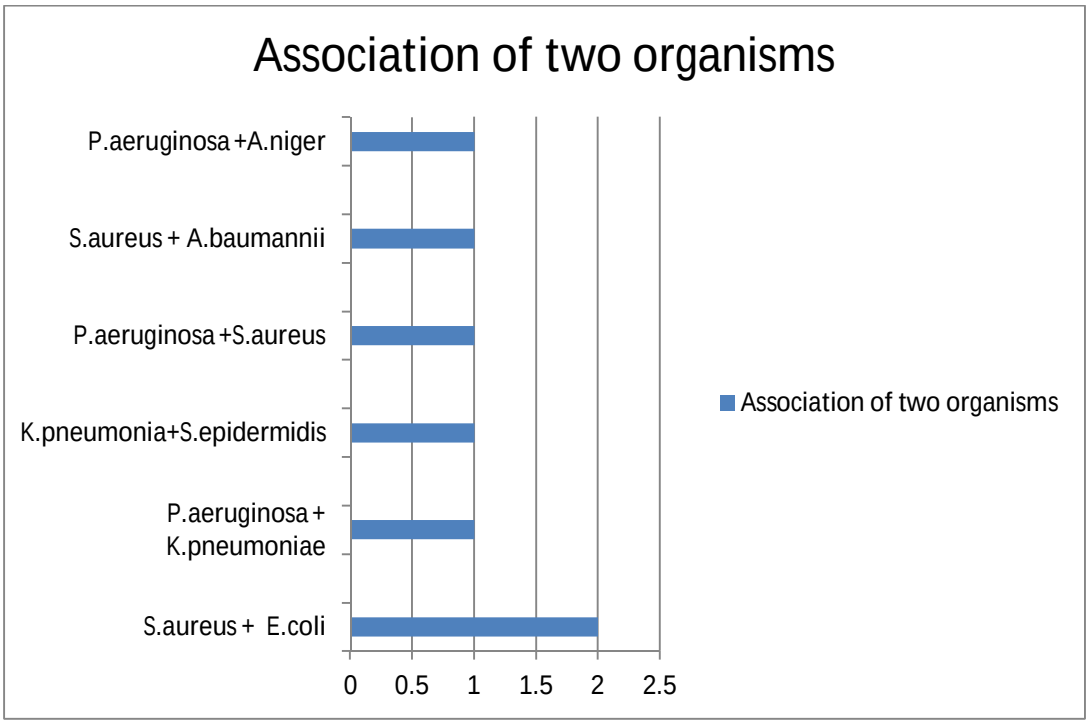


TABLE 13: ANTIMICROBIAL SUSCEBTIBILITY OF THE BACTERIAL ISOLATES

Antibiotic Sensitivity(S)	Number	Amikacin	Gentamicin	Ciprofloxacin	Piperacillin & Tazobactum	Ceftazidime	Cotrimoxazole	Cefotaxime	Penicillin	Erythromycin	Imipenem	Chloramphenicol
Pseudomonas aeruginosa	41	32	28	31	40	19	NT	NT	NT	NT	41	NT
Klebsiella pneumoniae	7	6	4	4	7	NT	3	1	NT	NT	NT	NT
Escherichia coli	5	4	4	1	5	NT	2	1	NT	NT	NT	NT
Proteus mirabilis	4	4	4	1	4	NT	1	NT	NT	NT	NT	NT
Acinetobacter baumannii	3	3	3	3	3	1	2	NT	NT	NT	1	NT
Staphylococcus aureus	32	29	NT	25	NT	NT	28	NT	10	20	NT	32
Staphylococcus epidermidis	5	5	0	4	NT	NT	2	NT	2	3	NT	5

TABLE 14.ANTOBIOTIC RESISTANCE PATTERN OF BACTERIAL ISOALATES

Antibiotic Resistance	Number	Amikacin	Gentamicin	Ciprofloxacin	Piperacillin & Tazobactum	Ceftazidime	Cotrimoxazole	Cefotaxime	Penicillin	Erythromycin	Imipenem	Chloramphenicol
Pseudomonas aeruginosa	41	9	13	10	1	22	NT	NT	NT	NT	0	NT
Klebsiella pneumoniae	7	1	3	3	NT	NT	4	6	NT	NT	NT	NT
Escherichia coli	5	1	1	4	NT	NT	2	4	NT	NT	NT	NT
Proteus mirabilis	4	0	0	3	0	NT	3	4	NT	NT	NT	NT
Acinetobacter baumannii	3	0	0	0	0	2	1	NT	NT	NT	NT	NT
Staphylococcus aureus	32	2	NT	7	NT	NT	3	NT	21	10	NT	0
Staphylococcus epidermidis	5	0	NT	1	NT	NT	3	NT	3	2	NT	0

TABLE 15 : ANTIBIOTIC PROFILE OF *Pseudomonas aeruginosa*

Pseudomonas aeruginosa N=41	Sensitive	%	Resistant	%	P value One Sample Z-Test
Amikacin	32	78.05	9	21.95	0.0001
Gentamicin	28	68.29	13	31.71	0.0062
Ciprofloxacin	31	75.61	10	24.39	0.0091
Piperacillin & Tazobactum	40	97.56	1	2.44	0.0001
Ceftazidime	19	46.34	22	53.66	0.5713
Imipenem	41	100.00	0	0.00	0.0001

FIG.11. ANTIBIOTIC PROFILE OF PSEUDOMONAS AERUGINOSA

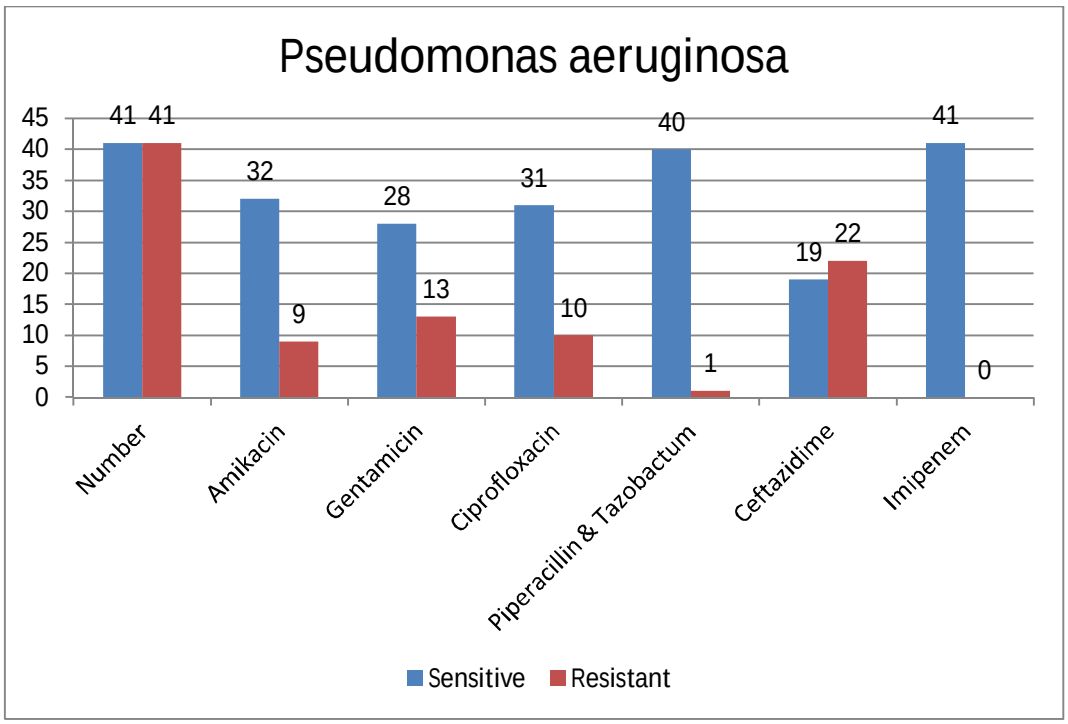


TABLE 16: ANTIBIOTIC PROFILE OF STAPHYLOCOCCUS AUREUS

Staphylococcus aureus N=32	Sensitive	%	Resistant	%	P value One Sample Z-Test
Amikacin	29	90.63	2	6.25	0.0001
Ciprofloxacin	25	78.13	7	21.88	0.0001
Cotrimoxazole	28	87.50	3	9.38	0.0001
Penicillin	10	31.25	21	65.63	0.0062
Erythromycin	20	62.50	10	31.25	0.0051
Chloramphenicol	32	100.00	0	0.00	0.0001

FIG.12. ANTIBIOTIC PROFILE OF STAPHYLOCOCCUS AUREUS

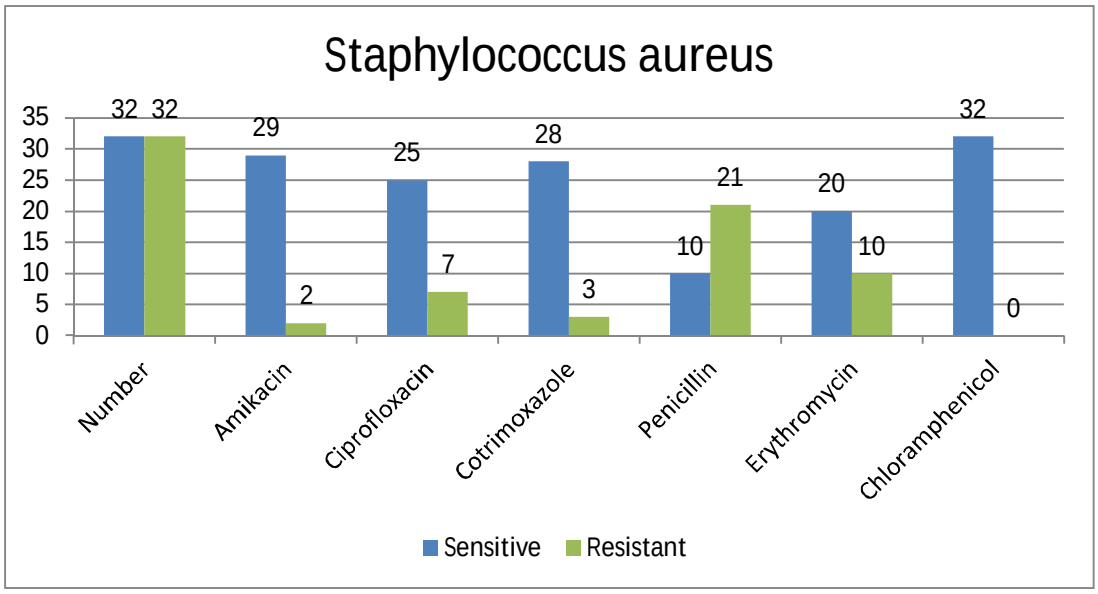


TABLE 17: ANTIMICROBIAL SUSCEBTIBILITY PATTERN OF KLEBSIELLA PNEUMONIAE

Klebsiella pneumonia N=7	Sensitive	%	Resistant	%	P value One Sample Z-Test
Amikacin	6	85.71	1	14.29	0.0001
Gentamicin	4	57.14	3	42.86	0.3210
Ciprofloxacin	4	57.14	3	42.86	0.3210
Piperacillin & Tazobactum	7	100.00	0	0.00	0.0001
Cotrimoxazole	3	42.86	4	57.14	0.3210
Cefotaxime	1	14.29	6	85.71	0.0001

FIG.13. ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF K. PNEUMONIAE

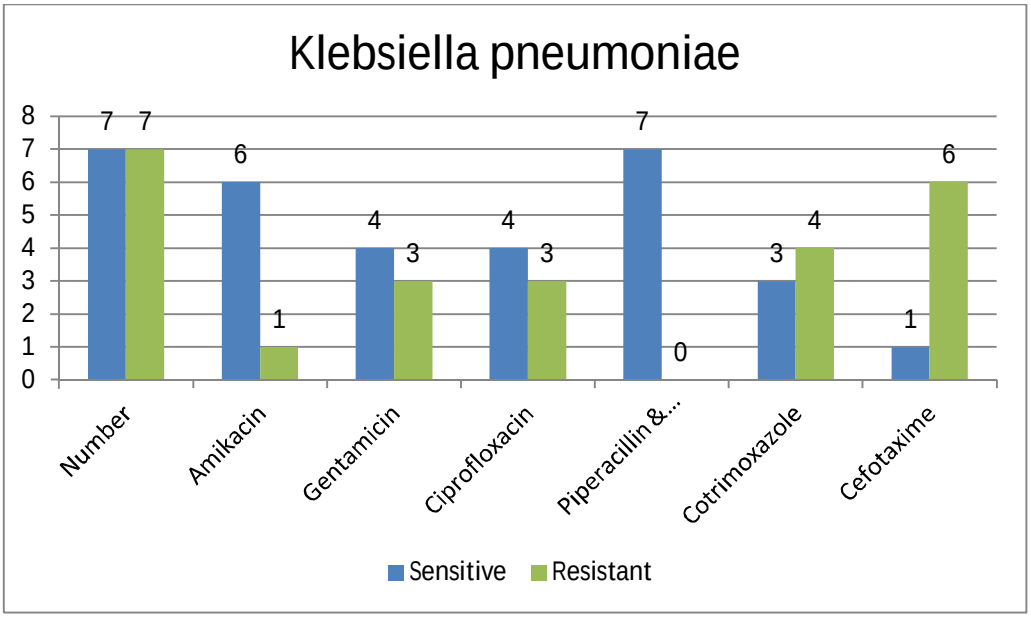


TABLE 18: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF E.COLI

Escherichia coli N=5	Sensitive	%	Resistant	%
Amikacin	4	80.00	1	20.00
Gentamicin	4	80.00	1	20.00
Ciprofloxacin	1	20.00	4	80.00
Piperacillin & Tazobactum	5	100.00	0	0.00
Cotrimoxazole	2	40.00	2	40.00
Cefotaxime	1	20.00	4	80.00

FIG 14: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF E.COLI

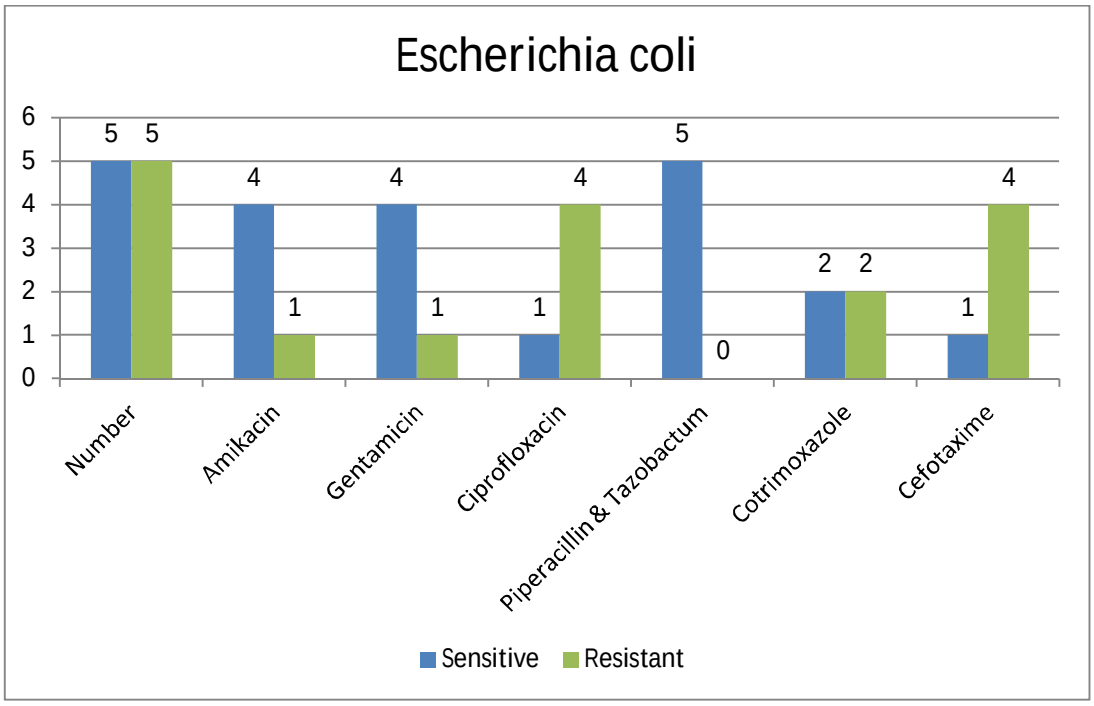


TABLE :19 ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF P.MIRABILIS

Proteus mirabilis N=4	Sensitive	%	Resistant	%
Amikacin	4	100.00	0	0.00
Gentamicin	4	100.00	0	0.00
Ciprofloxacin	1	25.00	3	75.00
Piperacillin & Tazobactum	4	100.00	0	0.00
Cotrimoxazole	1	25.00	3	75.00
Cefotaxime	0	0.00	4	100.00

FIG. 15. ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF P.MIRABILIS

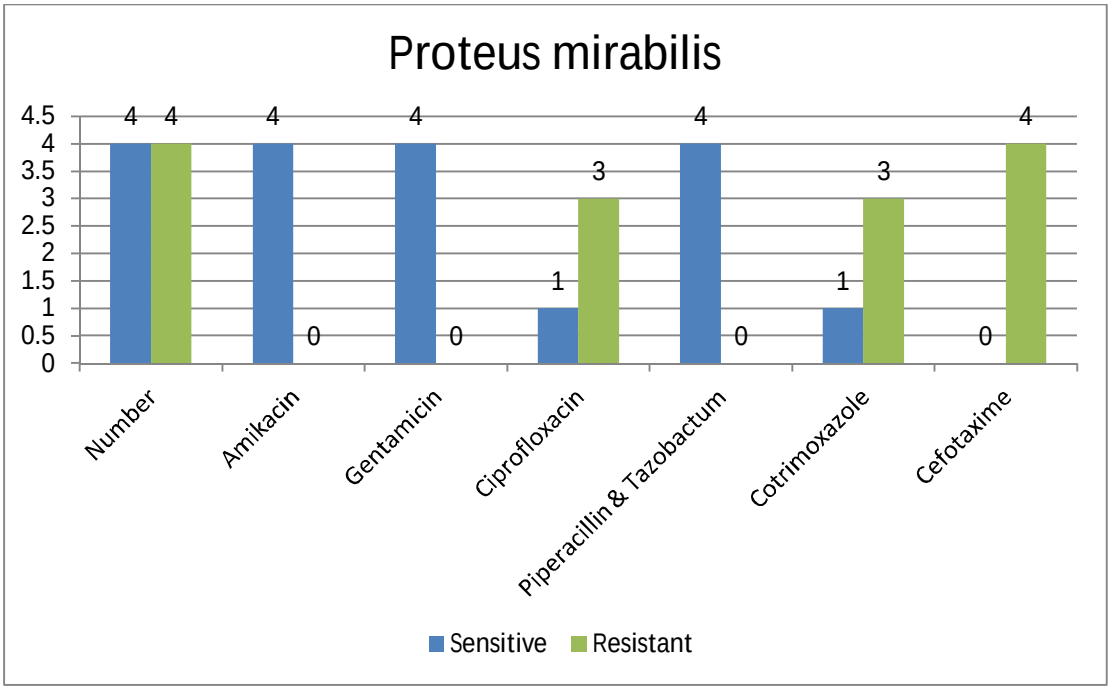


TABLE 20: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF A.BAUMANNII

Acinetobacter baumanni N=3	Sensitive	%	Resistant	%
Amikacin	3	100.00	0	0.00
Gentamicin	3	100.00	0	0.00
Ciprofloxacin	3	100.00	0	0.00
Piperacillin & Tazobactum	3	100.00	0	0.00
Ceftazidime	1	33.33	2	66.67
Cotrimoxazole	2	66.67	1	33.33

FIG.16. ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF A.BAUMANNII

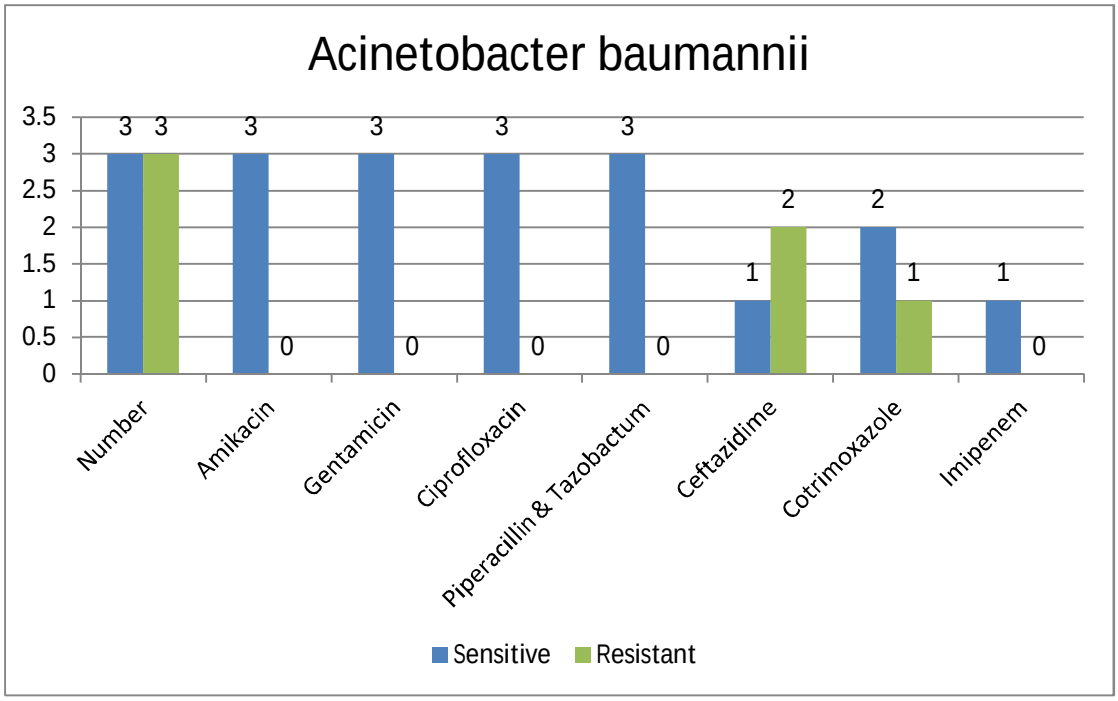


TABLE 21: DETECTION OF ESBL IN PSEUDOMONAS AERUGINOSA

ESBL Production	No of isolates	Percentage
Positive	7	17.07%
Negative	34	82.92%

TABLE 22: DETECTION OF AMPC BETALACTAMASE IN PSEUDOMONAS AERUGINOSA

AmpC Beta Lactamase	No.of Isolates	Percentage
Positive	4	9.75%
Negative	37	90.24%

TABLE 23: DETECTION OF MBL IN PSEUDOMONAS AERUGINOSA

Screening Test for MBL	No of Isolates	Percentage
Positive	0	0.00%
Negative	41	100%

DISCUSSION

Chronic Suppurative Otitis Media(CSOM) is considered as a major public health problem,in the developing world and India is one of the countries with high prevalence where urgent attention is needed.[1]

It is a persistent disease with risk of irreversible complications and is an important cause of preventable hearing loss in adults and children.[41]

Since chronic suppurative otitis media is a disease which can cause significant morbidity, early microbiological diagnosis ensures effective treatment.[42] Hence knowledge of pathogens and their antibiotic susceptibility pattern would guide the treating physicians in selection of appropriate antibiotics which would help us in reducing the complications and emergence of resistant strains.[43]

This cross sectional study which was conducted in the Institute of Microbiology in association with the Upgraded Institute of Otorhinolaryngology, Madras Medical College,Chennai, included 100 patients with clinical diagnosis of CSOM.

In the present study ,105 samples (pus from the middle ear) were obtained from 100 patients. Out of 100 patients, five had bilateral ear discharge accounting for 105 samples. Among 100 patients 93 were culture positive for aerobic bacteria and fungi.

Out of the five patients who had bilateral ear discharge- one patient had the same organism infecting both the ears and two had different organisms affecting each ear and two had growth in single ear .

Analysis of Age distribution showed peak incidence of CSOM in the second decade of life. Prakash et al in their study has reported peak incidence in the first decade of life.[8]

In the present study majority of patients had tubotympanic type of CSOM (88%)as compared to atticoantral type(12%). Sharma et al in their study has documented 72% of the cases as the safe or tubotympanic type and 28% as atticoantral type.

Monomicrobial growth was seen in 89 patients, whereas 7 showed polymicrobial growth .No growth was observed in 7 samples .

Aslam et al, in their study showed that out of 142 samples studied , 76% of them were pure and 23.9% were mixed cultures.[44] Poorey and lyer from India in their study isolated pure growth from 82, mixed growth from 10 samples out of 100 samples included.[45]

Analysis of bacterial profile of the present study showed that Gram negative bacilli outnumbered Gram positive cocci which is similar to studies by Naheed et al [51] and Kulchal et al[52]. The predominant pathogen was Pseudomonas aeruginosa (39.6%) , followed by Staphylococcus aureus (31.68%), Klebsiella pnuemoniae(6.8%), Escherichia coli(4.8%), and Proteus

mirabilis(3.8%)and Acinetobacter baumanii(2.9%). This is in concordance with many previous studies .[46,47,48,49,50] Shyamala *et al* have found that *Pseudomonas aeruginosa* and *Staphylococcus* species are the commonest organism isolated from otitis media.[46] In contrast, Prakash et al in their study has documented Staphylococcus aureus to be the predominant isolate comprising of 48.69% of the total followed by Pseudomonas aeruginosa.

These studies depict that the microbial profile vary between different regions,based on patient population and geographical distribution and hence necessitates the need for frequent analysis and update of the microbial profile in every region.

Pseudomonas aeruginosa, the predominant cause of CSOM in tropical region usually does not inhabit the upper respiratory tract, its presence in the middle ear cannot be ascribed to an invasion through Eustachian tube and it is considered to gain access to the middle ear via defect in TM.

Prakash et at[6] in their study reported Coliforms including Klebsiella pneumoniae and Escherichia coli in 9.42% and 7.33% cases respectively,and these findings were tandem to the study done by Mansoor et al.[57] who reported K.peumoniae in 8% and E.coli in 4% whereas Poorey and lyer[45] in their study has reported a high incidence of Klebsiella (25.4%).

A recent study by Shyamala and Reddy showed a different trend where *E. coli* was reported in 12% and Klebsiella in 5% of cases.[53] Frequent isolation of coliforms like Escherichia.coli and Klebsiella indicates that individuals are

at high risk of acquiring infection when they are exposed to water contaminated with faecal flora while bathing or in activities like swimming.

The Gram positive organisms isolated in the present study were Staphylococcus aureus (31%) and S.epidermidis(4.8%) which is similar to the studies done by Ettehad et al[68] and Singh et al[69] ,whereas Prakash et al has documented higher rate of isolation of Staphylococcus aureus(48.69%) in their study.

In the present study ,fungal culture was done in 25 patients based on the KOH mount and was positive in 6 patients.

Among the six fungal isolates , four were Aspergillus niger(66.67%) and two were Aspergillus fumigates(33.33%). In a study from Haryana, India, fungal etiology was found in 15% of cases, out of which 60% were Candida species and 40% were Aspergillus species.[54].Sen Gupta et al reported 74.2% of Aspergillus species and 19.3% of candida species among the fungal isolates. [55] In contast U Mohan et al in their study on 182 samples reported 25 fungal isolates of which 60%were Candida species and 40% were Aspergillus species.[56]

Antimicrobial Susceptibility

In the present study, antibiotic susceptibility testing was done for all the isolated organisms by Kirby-Bauer disc diffusion methodas per CLSI guideliness. Antibiotic susceptibility pattern of P.aeruginosa revealed 100%

susceptibility to Imipenem, while 97% of the isolates were sensitive to Piperazillin-tazobactum,78% to Amikacin,75% to Ciprofloxacin, 68%to Gentamicin and 46% to Ceftazidime.The sensitivity pattern of Pseudomonas aeruginosa was similar to the studies done by Gulati et al.,[58] and Mishra et al[59] and Lee et al [60] in which the authors have documented 100% susceptibility to Imipenem followed by Piperacillin-Tazobactum,Amikacin and Ciprofloxacin.

Mansoor T et al in their study showed that amikacin was active against 96% of the isolates of Pseudomonas, followed by ceftazidime (89%).[61]

Aminoglycosides are bactericidal antibiotics that interfere with protein synthesis and are frequently used because of its activity against Gram negative bacteria. In the present study majority of the Pseudomonas aeruginosa isolates were found to be more sensitive to amikacin than gentamicin and this finding is similar to previous studies in Sharma et al ,Nepal[62],Saini et al ,India [63]and Wario et al from Nigeria.[64]

Fluoroquinolones inhibits the bacterial DNA gyrase or the topoisomerase II thereby inhibiting DNA transcription and replication.They have a broad range of activity and found to be active against Pseudomonas aeruginosa.In the present study 75% of P.aeruginosa was found to be sensitive to ciprofloxacin which is similar to various studies that has reported 90% Sensitivity to ciprofloxacin.

Ceftazidime is a most frequently prescribed third generation cephalosporin that has an extended Gram negative spectrum. However resistance to ceftazidime is increasing ,complicating the management of patients with such isolates.

In the present study, 53% of isolates of P.aeruginosa were resistant to Ceftazidime which is in concordance to the study conduced by Sabiranatah et al[65] in which 54% of the Pseudomonas isolates showed 54% resistance to ceftazidime. Contrary to this , the study by chavan et al [66] has reported 84% sensitivity to ceftazidime.

Ceftazidime resistance is mainly mediated by production of β-lactamases such as ESBL, MBL and occasionally AmpC-β-lactamases [67] Besides production of various β-lactamases, other mechanisms such as the lack of drug penetration due to mutation in porins, loss of certain outer membrane proteins and efflux pumps may also contribute for resistance to β-lactams.

In the present study the rates of ESBL,Amp C and MBL were determined among the isolates of Pseudomonas resistant to ceftazidime.

In the present study out of 41 isolates of P.aeruginosa, [7] 17.07% were found to be positive for ESBL production. These observations suggest that the ESBLs which are generally widespread among members of Enterobacteriaceae are also increasingly found in P. aeruginosa. This is in concordance with the studies of Aggarwal et al and Picao et al who documented ESBL production of about 21% .[70,71]

ESBL producing organisms are frequently resistant to other classes of ntibiotics, including aminoglycosides and fluoroquinolones due to the coexistence of genes encoding drug resistance to other antibiotics on the plasmids which encode ESBL.

With regard to AmpC ßlactamases in the present study 9.75% of the P.aeruginosa isolates were observed to produce AmpC producer but the study done by Parveen et al, AmpC production was observed in 55.5% of the ceftazidime resistant Pseudomonas spp [(72)].

All the isolates of P.aeruginosa were sensitive to Imipenem and there were no MBL producers in the present study.

The second most commonly isolated organism in our study was Staphylococcus aureus comprising of 32% of the isolates. All the isolate of staphylococcus aureus were found to be methicillin sensitive(MSSA) where 90%of the isolates were sensitive to amikacin,87% to cotrimoxazole,78% to ciprofloxacin and 62% to Erythromycin.This correlates with the reports of the study done by Prakash et al where out of 181 isolates , 48% of isolates were Staphylococcus aureus and all were found to be methicillin sensitive.

In our study Escherichia coli showed 80% sensitivity with Amikacin 20% sensitivityto ciprofloxacin.The sensitivity of Proteus mirabilis was good with amikacin and gentamicin. Klebsiella pneumoniae showed 85% sensitivity with amikacin and 57% sensitivity with both ciprofloxacin, and gentamicin.

CSOM is generally treated by oral medications with quinolone antibiotic drops such as ciprofloxacin. The absence of oral formulations of Ceftazidime, Cefipime and Imipenem has severely limited the use of these antibiotics in patients with CSOM otorrhea, thus restricting the ability of these bacteria to develop resistance to these antibiotics. This may explain,why resistance to Imipenem in bacteria from CSOM otorrhea is lower than in other types of bacterial infection. [73,74]

Multidrug resistant in Pseudomonas aeruginosa is classified as resistance to more than three antimicrobial catagories.[75]

In the present study isolation rate of multidrug resistant Pseudomonas aeruginosa was found to be 9.75%.Similar results were obtained in the study by Lee et al.[76]

The present study has showed that CSOM infections are commonly caused by Pseudomonas aeruginosa followed by Staphylococcus aureus.However Pseudomonas infections are becoming less sensitive against commonly used antimicrobials like ciprofloxacin, cephalosporins, and gentamicin .Resistance to antimicrobial agents is an increasing public health threat. It limits therapeutic options and leads to increased morbidity and mortality. The important factor that is responsible for resistance is inappropriate use of antibiotics.It is essential to follow antibiotic policies which guides judicious and appropriate use antibiotics to prevent emergence and spread of resistant pathogens.

Hence Continuous and periodic evaluation of microbiological pattern and antimicrobial sensitivity of the isolates is necessary to decrease the risk of complications and emergence of resistant microorganisms by early institution of appropriate treatment.

SUMMARY

- A total of 100 patients with clinical diagnosis of CSOM from the Upgraded Institute of Otorhinolaryngology were included in the study.
- There was a male predominance among the cases(57%)
- Analysis of age distribution showed maximum no of patients with CSOM in the second decade of life.
- The risk factors associated include upper respiratory tract infection,allergy,tonsillitis and deviated nasal septum. Many patients had more than one risk factor associated with CSOM.
- Out of 100 patients ,88 patients had tubotympanic (safe) type of CSOM,and 12 had atticoantral type of CSOM.
- Analysis of culture results showed culture positive in 93 patients and no growth was observed in 7 patients.
- In the present study out of 100 patients ,95 patients had unilateral CSOM and 5 had ear discharge from both the ears.
- Monomicrobial growth was observed in 89 patients and polymicrobial in 7 patients.All the 5 patients who had bilateral CSOM had monomicrobial growth.One patient had the same organism infecting both the ears and two had different organisms affecting each ear and two had growth in single ear.

- Analysis of bacterial flora showed predominance of Pseudomonas aeruginosa (39.6%) followed by Staphylococcus aureus(31.68%).
- The other Gram negative isolates isolated were Klebsiella pneumonia(6.8%), E.coli,(4..8%). P.mirabilis(3.8%) and Acinetobacter baumannii(2.9%).
- The Gram Positive organisms isolated include Staphylococcus aureus(31.68%) and S.epidermidis(4.8%).
- In the present study fungal culture was done based on the KOH mount and was positive in 6 patients .Among the six fungal isolates,4 were A.niger and two were A.fumigatus.
- In the present study for all the isolates Amikacin was found to be the most effective drug followed by ciprofloxacin and gentamicin.
- Antimicrobial susceptibility of Pseudomonas aeruginosa revealed 100% sensitive to imipenem,97% sensitive to piperazillin-tazobactum,78% to amikacin ,75% to ciprofloxacin , 68% to gentamicin and 46% to ceftazidime.
- ESBL producers in Pseudomonas aeruginosa was found to be 17.07%,AmpC betalactamase was found in 9.75%
- There were no MBL producers as all the isolates of P.aeruginosa were sensitive to imipenem.
- Multidrug resistant Pseudomonas aeruginosa was found in 9.75%

CONCLUSION

Chronic suppurative otitis media is a major health problem in developing countries causing serious local damage and threatening complications.

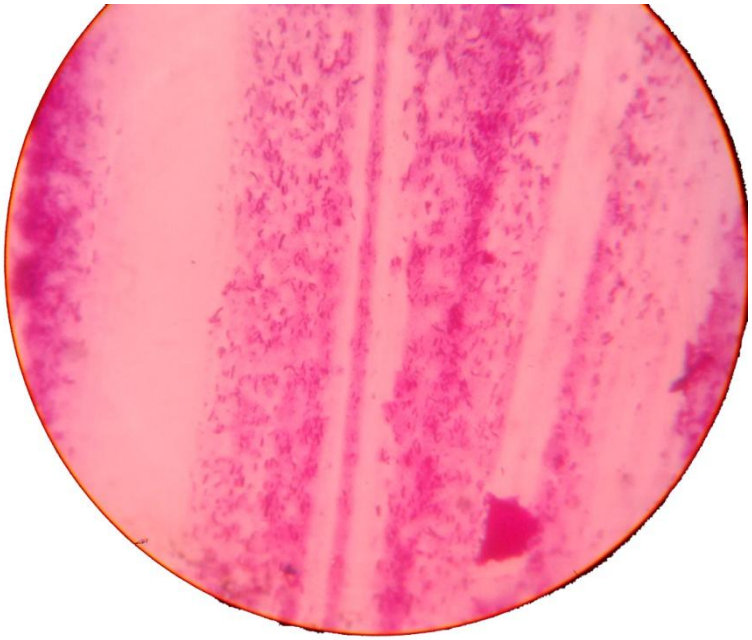
In the present study Pseudomonas aeruginosa was the major pathogen in the etiology of CSOM followed by staphylococcus aureus. These organisms are found to be less susceptible to the routinely used drugs like ciprofloxacin and cephalosporins. This may be due to important factor that the cultures are mostly requested when commonly used drugs have failed to eradicate the infection. . Hence appropriate antimicrobial drugs should be prescribed after proper diagnosis of the causative organism and its antimicrobial susceptibility pattern.

The results of this study indicates that ESBL and AmpC production is a major mechanism of resistance to cephalosporins among the isolates of P.aeruginosa.

The emergence of P. aeruginosa possessing combinations of β-lactamases like ESBL and AmpC betalactamases is a major public health concern necessitating efficient detection and intervention to control drug resistance.

Hence continuous and periodic evaluation of microbiological pattern and antibiotic sensitivity is essential to reduce the potential risk of complications and emergence of resistant strains.

DIRECT GRAM STAIN SHOWING GRAM NEGATIVE BACILLI



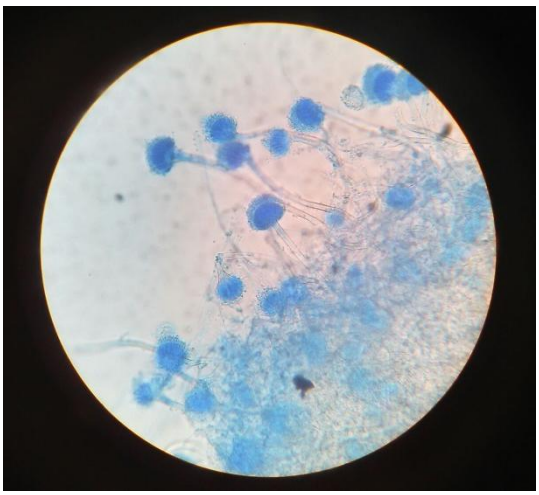
COLONIES OF PSEUDOMONAS AERUGINOSA ON NUTRIENT AGAR PLATE

COLONIES OF PSEUDOMONAS AERUGINOSA ON BLOOD AGAR PLATE

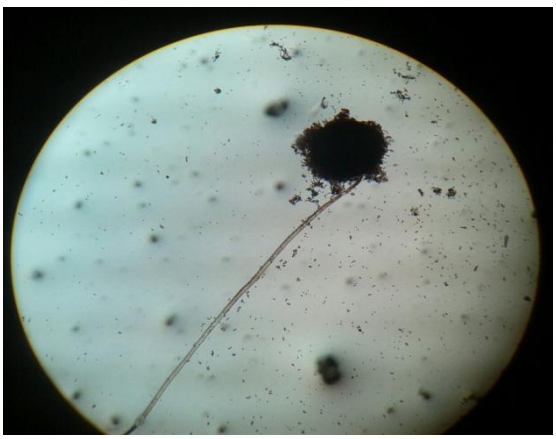
AMPC DISC TEST FOR AMPC BETALACTAMASE DETECTION

PHENOTYPIC CONFIRMATORY METHOD FOR ESBL

LPCB MOUNT OF ASPERGILLUS FUMIGATUS



LPCB MOUNT OF ASPERGILLUS NIGER



APPENDIX –I

ABBREVATIONS

CSOM	-	Chronic Suppurative Otitis Media
URTI	-	Upper Respiratory Tract Infection
DNS	-	Deviated Nasal Septum.
CLSI	-	Clinical & Laboratory Standards Institute
ATCC	-	American Type Culture Collections
GNB	-	Gram Negative Bacilli
GPC	-	Gram Positive Bacilli
MH broth	-	Mueller Hinton broth
MHA	-	Mueller Hinton Agar
MIC	-	Minimum Inhibitory Concentration
MRSA	-	Methicillin Resistant Staphylococcus aureus
MSSA	-	Methicillin Sensitive staphylococcus aureus
PCDDT	-	Phenotypic Confirmatory Disk Diffusion Test
DDST	-	Double Disc Synergy Test
ESBL	-	Extended Spectrum Beta Lactamase
MBL	-	Metallo BetaLactamase
MHT	-	Modified Hodge Test
LPCB	-	Lactophenol cotton blue
NT	-	Not tested
AK	-	Amikacin
GM	-	Gentamicin

CIP	-	Ciprofloxacin
PT	-	Piperacillin Tazobactum
CAZ	-	Ceftazidime
COT	-	Cotrimoxazole
CTX	-	Cefotaxime
PEN	-	Penicillin
ERY	-	Erythromycin
CX	-	Cefoxitin
IMP	-	Imipenem
CHLOR	-	Chloramphenicol

APPENDIX –II

A. STAINS AND REAGENTS

I. Gram staining

Methyl violet (2%)	l0g Methyl violet in l00ml absolute alcohol in 1litre of distilled water (primary stain)
Grams Iodine	l0g Iodine in 20g KI (fixative)
Acetone	Decolourising agent
Carbol fuchsin 1%	Secondary stain

II. 10% KOH

Potassium hydroxide	l0g
Glycerol	l0ml
Distilled water	80ml

III. Lactophenol cotton blue stain:

Lactic acid	20 ml
Phenol	20ml
Cotton blue (dye)	0.5g
Glycerol	40ml
Distilled water	20ml

B. MEDIA USED:

1. Nutrient agar

Peptic digest of animal tissue	5g
Sodium chloride	5g

Beef extract	1.5g
Yeast extract	1.5g
Agar	15gm
Final pH	7.4±0.2

Suspend 28 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely and sterilized by autoclaving at 15 lbs pressure (120°C) for 15 minutes.

2. Mac Conkey agar

Peptone	20g
Sodium taurocholate	5g
Distilled Water	1 ltr
Agar	20g
2% neutral red in 50% ethanol	3.5ml
10% lactose solution	l00mI

Dissolve peptone and taurocholate in water by heating. Add agar and dissolve it in steamer. Adjust pH to 7.5. Add lactose and neutral red shake well and mix.Heat in free steam (100°C) for 1 hour, then autoclave at 115°C for 15 minutes.

3. Blood agar (5% sheep blood agar)

Peptone	l0g
NaCl	5g
Distilled water	1 Ltr
Agar	10g

Dissolve ingredients in distilled water by boiling, and add 5% sheep blood(sterile) at 55°C adjust pH to 7.4.

4. Sabouraud's dextrose agar

Dextrose	40g
Peptone	l0g
Agar 20g	
Distilled water	l000ml

pH = 5.5

Sterilise by autoclaving at 121ºC for 20 min

5. Mueller Hinton Agar:

Ingredients	**Gms / Litre**
Beef, infusion	300.000
Casein acid hydrolysate	17.500
Starch	1.500
Agar	17.000

Final pH (at 25°C) 7.3±0.1

Suspend 38 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Cool to 50o C .Add aseptically 5ml of sterile sheep blood to 95 ml of the agar. Mix well and dispense in aseptically into sterile petri dish.

B. MEDIA REQUIRED FOR BIOCHEMICAL IDENTIFICATION:

1. Oxidase Reagent

Tetra methyl p-phenylene diamine dihyrochloride- 1% aqueous solution.

2. Catalase

3% hydrogen peroxide

3. Indole test

Kovac's reagent

Amyl or isoamyl alcohol	150ml
Para dimethyl amino benzaldehyde	10g
Concentrated hydrochloric acid	50ml

Dissolve the aldehyde in the alcohol and slowly add the acid. Prepare in small quantities and store in the refrigerator. Shake gently before use.

4. Christensen's Urease test medium

Peptone	1g
Sodium chloride	5g
Dipotassium hydrogen phosphate	2g
Phenol red	6ml
Agar	20g
Distilled water	1 ltr
10% sterile solution of glucose	l0ml
Sterile 20% urea solution	l00ml

Sterilize the glucose and urea solutions by filtration. Prepare the basal medium without glucose and urea, adjust to pH 6.8-6.9 and sterilize by autoclaving in a flask at 121°C for 30min. Cool to about 50°C, add the glucose & urea, and tube the

medium as slopes.

5. Simmon's Citrate Medium

Koser's medium	1 ltr
Agar	20g
Bromothymol blue	0.2% 40ml

Dispense, autoclave at 121°C for 15 min and allow to set as slopes

6. Triple Sugar Iron medium

Beef extract	3g
Yeast extract	3g
Peptone	20g
Glucose	1g
Lactose	l0g
Sucrose	l0g
Ferric citrate	0.3g
Sodium chloride	5g
Sodum thiosulphate	0.3g
Agar	12g
Phenol red 0.2% solution	l2ml
Distilled water	1 ltr

Heat to dissolve the solids, add the indicator solution, mix and tube. Sterilize at 121°C for 15 min and cool to form slopes with deep butts.

7. Glucose phosphate broth

Peptone	5g
Dipotassium hydrogen phosphate	5g
Water	1 ltr
Glucose 10% solution	50ml

Dissolve the peptone and phosphate and adjust the pH to 7.6. Filter dispense in 5ml amounts and sterilize at 121°C for 15min. Sterilize the glucose solution by filtration and add 0.25ml to each tube

Methyl Red Reagent

Methyl Red	10mg
Ethyl alcohol	30ml
Distilled water	20ml

Voges Proskauer Reagent

Reagent A:

Alpha naphthol	5g
Ethyl alcohol	100ml

Reagent B:

Potassium hydroxide	40g
Distilled water	100ml

8. Peptone water fermentation test medium.

To the basal medium of peptone water, add sterilised sugars of 1% indicator :bromothymol blue with Durham's tube.

Basal medium peptone water

Sugar solutions:

Sugar	1ml
Dislilled water	l00ml

pH = 7.6.

9. Mannitol motility medium

Agar	5g
Peptone	1g
Potassium nitrate	1g
Mannitol	2g

Phenol red indicator

Distilled water l000ml

pH 7.2

11. Potassium nitrate broth

Potassium nitrate (KN03)	0.2gm
Peptone	5.0gm
Distilled water	100ml

The above ingredients were mixed and transferred into tubes in 5 ml amount and autoclave.

12. 2% Sodium deoxycholate solution:

Ingredients :

Sodium deoxycholate	2 gms
Distilled water	100ml

Dissolve 2 gms of deoxycholate in 100 ml of distilled water .Mix well.Store in a sterile containers.

8. Decarboxylase media:

8a. Moller decarboxylase broth base:

Ingredients	**gms/L**
Peptone	5
Beef extract	5
Bromocresol purple	0.01
Cresol red	0.005
Glucose	0.5
Pyridoxal	0.005

Final pH 6

8b. Aminoacid:

Add 10 g of the levo form of the aminoacid for 1000ml.mix and dispense in sterile tubes.

9. Hugh & Leifson's Oxidation –Fermentation test:

Peptone	2g
Sodium chloride	5g
D-glucose	10g
Bromothymol blue	0.03g
Agar	3.0g
Dipotassium phosphate	0.30g
Distilled water	1L

pH =7.1

Basal medium is autoclaved.1% of sterile sugar solutions is added to the basal medium.Dispense into sterile test tubes without slant.

ANNEXURE –I

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No. 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. Sangeetha Baskaran,
Postgraduate M.D.(Microbiology),
Madras Medical College,
Chennai – 600 003.

Dear Dr.Sangeetha Baskaran,

The Institutional Ethics Committee has considered your request and approved your study titled **"Evaluation of bacterial and fungal etiological agents of chronic suppurative otitis media with special reference to antimicrobial resistance pattern of pseudomonas species". No.19102014.**

The following members of Ethics Committee were present in the meeting held on 07.10.2014 conducted at Madras Medical College, Chennai-3.

1.	Dr.C.Rajendran, M.D.,	: Chairperson
2.	Dr.R.Vimala, M.D., Dean, MMC, Ch-3	: Deputy Chairperson
3.	Prof.B.Kalaiselvi, M.D., Vice-Principal, MMC, Ch-3	: Member Secretary
4.	Prof.R.Nandhini, M.D., Inst.of Pharmacology, MMC	: Member
5.	Prof.K.Ramadevi, Director i/c, Inst.of Biochemistry, MMC	: Member
6.	Prof.Saraswathy, M.D., Director, Pathology, MMC, Ch-3	: Member
7.	Prof.S.G.Sivachidambaram, M.D., Director i/c, Inst.of Internal Medicine, MMC	: Member
8.	Thiru S.Rameshkumar, Administrative Officer	: Lay Person
9.	Thiru S.Govindasamy, B.A., B.L.,	: Lawyer
10.	Tmt.Arnold Saulina, M.A., MSW.,	: Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI - 600 003

ANNEXURE –II

PROFORMA

Name : Age:

Sex:

Occupation:

Address:

Presenting complaints:

- Ear discharge R/L/Both
- Difficulty in Hearing
- Ear Pain
- Ringing of ear
- Fever
- Vomiting
- headaches

History of presenting illness:

Past history:

Personal history:

Habits

Treatment History:

General physical examination:

Local examination:

Systemic examination:

Provisional diagnosis:

Investigations

X ray

Mastoids

Lateral View of the Face

Paranasal Sinus

CT scan findings:

Microbiological Investigations:

Direct examination:

Gram stain-

KOH mount-

Bacterial Culture:

- NA
- MAC
- BAP

Fungal culture:

- SDA

Isolate identified in sample:

Antibacterial susceptibility pattern:

ANNEXURE –III

CONSENT FORM

STUDY TITLE

Evaluation of bacterial and fungal agents in the aetiology of chronic suppurative otitis media with special reference to antimicrobial resistance pattern of Pseudomonas species"

I……, hereby give consent to participate in the study conducted by Dr.SangeethaBaskaran, Post graduate at Institute of Microbiology, Madras Medical College, Chennai and to use my personal clinical data and the result of investigations for the purpose of analysis and to study the nature of the disease.

I have read and understood this consent form and the information provided to me.

I have had the consent document explained to me.

I have been explained about the nature of the study.

I have been explained about my rights and responsibilities by the investigator.

I have been informed the investigator of all the treatments I am taking or have taken in the past.

I have not participated in any research study within the past _____ month(s).

I am aware of the fact that I can opt out of the study at any time without having to give my reason and this will not affect my future treatment in this hospital.

I am also aware that the investigator may terminate my participation in the study at any time, for any reason, without any consent.

I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.

1. I have understand that my identity will be kept confidential if my data are publicly presented.
2. I have had my questions answered to my satisfaction.
3. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

I also give consent to give my clinical sample(Pus) for further investigations. I also learn that there is no additional risk in this study.

Name and signature / thumb impression of the participant

Name _____

Signature_____

Date_____

Name and Signature of the investigator

Name _____

Signature_____

Date_____

ANNEXURE –IV

Master CHART

S NO	AGE	SEX	EAR-R/L/B	ASSOCIATED FEATURES				Type of CSOM	Species Identified	ANTIBIOTIC SUSCEPTIBITITY TESTING											RESISTANT PATTERN
				URTI	ALLERGY	TONSILLITIS	DNS			AK	GM	CIP	PT	CAZ	COT	CTX	PEN	ERY	IMP	CHLOR	
1	57	F	R	+	_	_	_	AT	P.aeruginosa	S	S	S	S	S	_	_	_	_	S	_	
2	26	F	L	_	_	_	+	TT	P.aeruginosa	R	R	R	S	S	_	_	_	_	S	_	
3	22	M	B-R	+	_	+	_	AT	P.aeruginosa	S	S	S	S	R	_	_	_	_	S	_	
			B-L						S.aureus	S	_	S	_	_	S	_	R	S	_	S	
4	55	M	L	_	_	_	_	TT	P.aeruginosa	S	S	S	S	R	_	_	_	_	S	_	
5	18	F	R	_	+	_	_	TT	K.pneumonia	S	R	S	S	_	R	R	_	_	_	_	ESBL
6	25	F	L	+	_	_	+	TT	NG												
7	56	M	R	_	_	_	+	TT	S.aureus	S	_	S	_	_	_	_	R	R	_	S	
8	38	F	L	+	_	_	+	TT	P.aeruginosa	R	R	R	S	R	_	_	_	_	S	_	AmpC
9	21	F	R	_	_	_	+	TT	S.aureus	S	_	R	_	_	S	_	S	R	_	S	
10	45	F	R	+	_	_	+	AT	P.aeruginosa	S	S	R	S	S	_	_	_	_	S	_	
11	57	F	B-R	_	_	_	+	TT	K.pneumonia	S	R	R	S	_	R	R	_	_	_	_	ESBL
			B-L						NG												
12	78	M	R	+	_	_	_	TT	S.aureus	S	_	S	_	_	S	_	R	R	_	S	
13	45	F	R	_	_	+	+	TT	S.aureus	S	_	S	_	_	S	_	R	R	_	S	
14	21	M	R	+	+	_	_	TT	E.coli	S	S	S	S	_	R	R	_		_	_	ESBL
									S.aureus	S	_	S	_	_	S	_	R	S	_	S	
15	52	F	R	_	_	_	+	TT	NG												
16	40	F	R	+	_	_	_	TT	P.aeruginosa	S	R	S	S	R	_	_	_	_	S	_	AmpC

S NO	AGE	SEX	EAR-R/L/B	ASSOCIATED FEATURES				Type of CSOM	Species Identified	ANTIBIOTIC SUSCEPTIBITITY TESTING											RESISTANT PATTERN
				URTI	ALLERGY	TONSILLITIS	DNS			AK	GM	CIP	PT	CAZ	COT	CTX	PEN	ERY	IMP	CHLOR	
17	31	F	R	+	+	_	_	TT	P.aeruginosa	S	R	S	S	R	_	_	_	_	S	_	AmpC
									K.pneumonia	S	S	R	S	_	R	R	_	_	_	_	ESBL
18	60	M	B-R	+	_	_	+	TT	S.aureus	S	_	S	_	_	S	_	R	S	_	S	
			B-L						A.baumannii	S	S	S	S	S	S	_	_	_	_	_	
19	32	M	R	_	_	_	_	TT	P.aeruginosa	R	R	S	S	R	_	_	_	_	S	_	
20	39	F	L					TT	CONS	S	_	S	_	_	S	_	R	R	_	S	
									K.pneumonia	S	S	S	S	_	S	R	_	_	_	_	ESBL
21	25	M	L	_	+	_	_	AT	P.aeruginosa	S	S	S	S	R	_	_	_	_	S	_	
									A.niger												
22	30	M	B-R	_	_	_	_	TT	S.aureus	S	-	R	_	_	S	_	R	S	_	S	
			L						NG												
23	49	M	R	+	_	_	_	TT	K.pneumonia	R	R	R	S	_	R	R	_	_	_	_	ESBL
24	23	M	L	_	_	_	+	TT	S.aureus	S	_	S	_	_	S	_	R	S	_	S	
25	29	F	L	_	_	_	_	TT	S.aureus	S	_	S	_	_	R	_	R	R	_	S	
26	52	F	L	_	_	_	_	TT	A.niger												
27	41	M	L	+	_	_	_	TT	NG												
28	32	M	R	_	+	_	+	TT	S.aureus	S	_	S	_	_	S	_	S	S	_	S	
									P.aeruginosa	S	S	S	S	S	_	_	_	_	S	_	
29	39	M	R	_	_	_	+	TT	S.aureus	S	_	S	_	_	S	_	S	S	_	S	
30	48	F	B-R	+	_	_	_	AT	P.aeruginosa	S	S	S	S	S	_	_	_	_	S	_	
			B-L						P.aeruginosa	S	S	S	S	S	_	_	_	_	S	_	
31	21	M	R	+	_	+	_	TT	P.aeruginosa	S	S	S	S	S	_	_	_	_	S	_	

S NO	AGE	SEX	EAR-R/L/B	ASSOCIATED FEATURES				Type of CSOM	Species Identified	ANTIBIOTIC SUSCEPTIBITITY TESTING											RESISTANT PATTERN
				URTI	ALLERGY	TONSILLITIS	DNS			AK	GM	CIP	PT	CAZ	COT	CTX	PEN	ERY	IMP	CHLOR	
32	26	M	R	_	_	_	+	TT	A.fumigatus												
33	20	M	L	_	_	+	_	TT	S.aureus	S	_	R	_	_	S	_	R	R	_	S	
34	65	M	R	_	_	_	+	TT	A.niger												
35	39	M	R	_	_	+	_	AT	P.aeruginosa	S	S	S	S	S					S	_	
36	23	F	R	+	_	_	_	AT	P.aeruginosa	R	R	S	S	R	_	_	_	_	S	_	ESBL
37	23	F	R	_	_	_	+	TT	S.aureus	R		R	_	_	S	_	S	S	_	S	
38	13	M	R	+	_	+	_	TT	NG												
39	38	F	R	_	_	_	_	TT	P.aeruginosa	S	S	S	S	R	_	_	_	_	S	_	
40	25	F	L	+	_	_	_	TT	P.aeruginosa	S	S	S	S	S	_	_	_	_	S	_	
41	27	F	L	_	_	_	+	TT	P.aeruginosa	S	S	S	S	S	_	_	_	_	S	_	
42	48	F	R	+	_	_	+	TT	P.aeruginosa	R	R	S	S	R	_	_	_	_	S	_	ESBL
43	20	M	L	_	+	_	_	TT	P.aeruginosa	S	S	S	S	R	_	_	_	_	S	_	
44	30	F	L	_	+	_	+	AT	P.aeruginosa	S	S	S	S	S	_	_	_	_	S	_	
45	35	F	R	_	_	_	_	TT	P.aeruginosa	R	R	S	S	R	_	_	_	_	S	_	ESBL
46	14	M	R	_	+	_	+	TT	E.coli	S	S	R	S	_	_	R	_	_	_	_	ESBL
47	34	F	L	_	_	_	_	TT	P.aeruginosa	R	R	S	S	S	_	_	_	_	S	_	
48	58	M	L	_	_	_	+	AT	P.aeruginosa	S	S	S	S	R	_	_	_	_	S	_	
49	63	M	L	+	_	_	_	TT	P.aeruginosa	S	R	S	S	R	_	_	_	_	S	_	
50	35	F	L	_	+	_	_	TT	S.aureus	S	_	S	_	_	S	_	R	S	_	S	
51	15	F	L	+	_	+	+	TT	NG												
52	21	F	L	_	+	+	_	TT	P.aeruginosa	S	S	R	S	R	_	_	_	_	S	_	
53	50	M	L	_	_	_	_	TT	P.aeruginosa	S	S	S	S	R	_	_	_	_	S	_	

S NO	AGE	SEX	EAR-R/L/B	ASSOCIATED FEATURES				Type of CSOM	Species Identified	ANTIBIOTIC SUSCEPTIBITITY TESTING											RESISTANT PATTERN
				URTI	ALLERGY	TONSILLITIS	DNS			AK	GM	CIP	PT	CAZ	COT	CTX	PEN	ERY	IMP	CHLOR	
54	64	F	R	+	_	_	_	AT	P.aeruginosa	S	S	S	S	S	_	_	_	_	S	_	
55	23	M	R	+	_	+	+	TT	CONS	S	_	S	_	_	S	_	R	S	_	S	
56	22	M	R	_	_	_	+	TT	P.aeruginosa	R	R	R	S	S	_				S	_	
57	60	M	R	+	_	_	+	TT	S.aureus	_	_	S	_	_	S	_	R	R	_	S	
58	34	F	L	_	_	_	_	TT	S.aureus	S	_	R	_	_	S	_	R	_	_	S	
									E.coli	S	S	R	S	_	S	R	_	_	_	_	ESBL
59	37	M	L	_	_	_	_	AT	P.aeruginosa	R	R	R	S	R	_	_	_	_	S	_	
60	37	M	R	+	_	_	_	TT	E.coli	S	S	R	S	_	S	S	_	_	_	_	
61	35	M	L	_	_	_	+	TT	Proteus mirabilis	S	S	S	S	_	R	R	_	_	_	_	ESBL
62	55	F	L	_	_	_	_	TT	S.aureus	S	_	S	_	_	S	_	R	S	_	S	
63	22	M	R	+	+	_	+	TT	S.aureus	S	_	S	_	_	S	_	S	S	_	S	
64	68	M	R	+	_	_	_	TT	CONS	S	_	S	_	_	R	_	S	S	_	S	
65	40	M	R	_	_	_	_	TT	E.coli	R	R	R	S	_	R	S	_	_	_	_	
66	36	M	L	_	+	_	_	TT	S.aureus	S	_	R	_	_	R	_	R	R	_	S	
67	35	M	R	+	_	+	+	TT	P.aeruginosa	S	S	R	S	R	_	_	_	_	S	_	ESBL
68	67	M	R	+	_	_	_	TT	S.aureus	S	_	S	_	_	S	_	S	S	_	S	
69	38	M	L	+	+	_	_	TT	Proteus mirabilis	S	S	R	S	_	R	R	_	_	_	_	
70	47	F	R	_	_	_	_	TT	P.aeruginosa	S	S	S	S	S	_	_	_	_	S		
71	46	M	R	_	_	_	+	TT	S.aureus	S	_	S	_	_	S	_	S	S	_	S	
72	56	F	R	+	_	_	_	TT	P.aeruginosa	S	S	S	S	S	_	_	_	_	S	_	
73	27	M	R	_	+	+	+	TT	S.aureus	S	_	S	_	_	S	_	R	S	_	S	

S NO	AGE	SEX	EAR-R/L/B	ASSOCIATED FEATURES				Type of CSOM	Species Identified	ANTIBIOTIC SUSCEPTIBITITY TESTING											RESISTANT PATTERN
				URTI	ALLERGY	TONSILLITIS	DNS			AK	GM	CIP	PT	CAZ	COT	CTX	PEN	ERY	IMP	CHLOR	
74	49	M	R	+	_	_	_	TT	S.aureus	S	_	S	_	_	S	_	R	S	_	S	
75	67	M	R	_	_	_	+	TT	S.aureus	S	_	S	_	_	S	_	R	R	_	S	
76	60	M	R	+	_	_	_	TT	S.aureus	R	_	R	_	_	R	_	R	R	_	S	
77	55	M	L	+	_	+	_	TT	CONS	S	_	R	_	_	R	_	R	R	_	S	
78	34	F	L	_	_	_	_	TT	A.niger												
79	55	F	R	_	_	_	+	TT	S.aureus	S	_	S	_	_	S	_	R	S	_	S	
80	22	M	L	+	+	_	+	TT	S.aureus	S	_	S	_	_	S	_	S	S	_	S	
81	56	M	L	_	_	_	_	TT	P.aeruginosa	S	S	S	S	S	_	_	_	_	S	_	
82	25	M	L	_	_	_	+	TT	S.aureus	S	_	S	_	_	S	_	S	S	_	S	
83	19	M	L	_	+	_	_	TT	NG												
84	45	F	L	_	_	_	_	TT	A.baumannii	S	S	S	S	R	R	_	_	_	_	_	
85	50	M	L	_	_	_	_	TT	Proteus mirabilis	S	S	R	S	_	R	R	_	_	_	_	ESBL
86	45	F	R	+	_	_	_	TT	K.pneumonia	S	S	S	S		S	R	_	_	_	_	ESBL
87	30	F	L	+	_	_	_	TT	K.pneumonia	S	S	S	S		S	S	_	_	_	_	
88	50	F	R	_	_	_	_	TT	P.mirabilis	S	S	R	S		S	R	_	_	_	_	ESBL
89	25	M	R	+	+	_	+	TT	NG												
90	63	M	L	+	_	_	_	TT	A.baumannii	S	S	S	S	R	S	_	_	_	S	_	
									CONS	S	_	S	_	_	R	_	S	S	_	S	
91	46	M	R	_	_	+	+	TT	P.aeruginosa	S	S	S	S	S	_	_	_	_	S	_	
92	28	F	R	+	+	+	+	TT	P.aeruginosa	S	S	S	S	S	_	_	_	_	S	_	
93	37	F	L	_	_	_	+	AT	P.aeruginosa	S	S	R	S	R	_	_	_	_	S	_	
94	65	M	R	_	_	_	_	TT	P.aeruginosa	S	S	S	R	R	_	_	_	_	S	_	

S NO	AGE	SEX	EAR-R/L/B	ASSOCIATED FEATURES				Type of CSOM	Species Identified	ANTIBIOTIC SUSCEPTIBITITY TESTING											RESISTANT PATTERN
				URTI	ALLERGY	TONSILLITIS	DNS			AK	GM	CIP	PT	CAZ	COT	CTX	PEN	ERY	IMP	CHLOR	
95	55	F	R	+	_	_	+	TT	A.fumigatus												
96	36	M	R	_	_	_	+	TT	S.aureus	S	_	S	_	_	S	_	S	S	_	S	
97	45	F	R	+	_	_	+	TT	P.aeruginosa	S	R	R	S	R	_	_	_	_	S	_	ESBL
98	13	M	L	_	_	+	+	TT	S.aureus	S	_	S	_	_	S	_	_	_	_	S	
99	35	M	R	_	_	_	_	TT	S.aureus	S	_	S	_	_	S	_	R	S	_	S	
100	70	M	R	+	_	_	+	TT	P.aeruginosa	S	S	R	S	R	_	_	_	_	S	_	AmpC

AK	AMIKACIN
GM	GENTAMICIN
CIP	CIPROFLOXACIN
PT	PIPERACILLIN TAZOBACTUM
CAZ	CEFTAZIDIME
COT	COTRIMOXAZOLE
CTX	CEFOTAXIME
PEN	PENICILLIN
ERY	ERYTHROMYCIN
CX	CEFOXITIN
IMP	IMIPENEM
CHLOR	CHLORAMPHENICOL

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